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ADVANCES IN LEGUME BIOLOGY

Edited by C. H. Stirton & J. L. Zarucchi

Monographs in Systematic Botany
from the Missouri Botanical Garden

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C. H. Stirton and J. L. Zarucchi

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This volume is dedicated to the memory of

P. J. M. BRENAN

former Director of the Royal Botanic Gardens, Kew,
and long-time researcher of the Legume Family.

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INTRODUCTION

The Second International Legume Conference, sponsored jointly by the Missouri Botanical Garden and the Royal Botanic Gardens, Kew, was held in St. Louis, Missouri, 23–27 June 1986. The Conference was attended by approximately 230 people from 26 countries with both invited papers and more than 100 poster papers presented on a wide variety of topics concerning the biology of the legume family. Upon an earlier unanimous vote by its participants, plans for this Second International Legume Conference began developing soon after the first Conference, which was held during the summer of 1978 at Kew Gardens in England. *Advances in Legume Systematics* (R. M. Polhill & P. H. Raven, eds.–1981) and *Advances in Legume Science* (R. J. Summerfield & A. H. Bunting, eds.–1980) are the published results of that successful two-week gathering of systematists, agronomists, and other biologists, meeting to share knowledge about this third-largest and economically important family of plants. In 1986 the agronomic counterpart to the St. Louis Conference was convened 6–11 July in Spokane, Washington, and focused on four cool season legume crops: pea, lentil, faba bean, and chickpea. The proceedings volume from that conference, *World Crops: Cool Season Food Legumes* (R. J. Summerfield, ed.), appeared in 1988.

In preparing for the 1986 Conference in St. Louis, the Organizing Committee (Peter H. Raven, Roger M. Polhill, Charles H. Stirton, and James L. Zarucchi) considered it best to develop the program focusing on current advances being made in our understanding of legume biology. The program was specifically designed by C. H. Stirton so that the papers and presentations communicated results of both field and experimental research, covering pure as well as applied points of view. More than an ad hoc assemblage of papers, the program was to be synthetic and modular, ranging from the microscopic to the organismic, yet be concerned with function and structure set in a phylogenetic framework; underlying all of this was the attempt to be as multidisciplinary as possible. Naturally, due to the constraints of time it was impossible to devote great depth to any one aspect of legume biology, whereas each area covered could itself serve as the focus for a conference of similar or longer duration. The program was designed to commence with a brief overview, followed by the discussion of the structure and function of organ systems. Beginning with overall plant architecture and then moving through each of its biological modules, paper presentations and scheduled discussion periods also covered the possible interaction of such modules with other organisms.

With just a brief scan of the table of contents on the pages immediately preceding, it is evident that the papers published herein cover a broad range of topics in legume biology with a wide variety of approaches to the subject matter. Plant growth, architecture, and the resulting forms that organisms may acquire begin this volume and present an interesting structure from which we may view other aspects of legume biology. The close study of growth and the life history of *Trifolium repens* (Sackville Hamilton) and the overview of architectural types encountered in the Leguminosae (Oldeman) provide focused and broad views in studying living organisms. As with the modular approach used in interpreting plant form, studies on inflorescences (Weberling), floral organogenesis (Tucker), pollen evolution (Guinet & Ferguson), and the question of pollen–stigma–style interactions (Owens & Stirton, Owens, Kenrick & Knox, and Shivanna & Owens) address these individual topics in a manner that provides an overview of the incredible variation found in legumes, yet yield detailed and exciting observations of unusual or previously little-studied species.

The complex subject of pollination, citing numerous species of Leguminosae as examples, is presented in several ways in this volume. A multidisciplinary approach to pollination biology (Schrire), with a very extensive appendix, provides a suggested framework of guidelines to be used

in studying the subject. The chapter following, covering seed:ovule ratios and mating systems in members of the three legume subfamilies (Bawa & Buckley), highlights the topics of seed abortion and microgametophytic selection in representative tropical species. With a narrowing of phylogenetic focus, the floral ecology of Australian acacias (Bernhardt) presents results of personal field observations of pollination in this diverse genus of mimosoid legumes. The two following papers each deal with a single legume species: the population biology and gene flow in *Lupinus texensis* (Schaal) and the natural history of *Ateleia herbert-smithii* (Janzen) provide results of two detailed, in-depth studies of two representative papilionoid legumes. The simple message we receive from these and other chapters is that for studies on leguminous plants (as well as for most organisms), much is known about a very few species and next to nothing concerning the vast majority. This is particularly alarming in light of the rapidly accelerating rate of deforestation in the tropics.

The chapter covering ecological success in relation to plant form and function in the woody legumes (Rundel) points out the degree of dominance and diversity of the family. From deserts to evergreen rainforests, legumes occur in virtually every terrestrial ecosystem. This broad overview of the physiological and morphological adaptations resulting in the evolutionary success of the Leguminosae, leads to the three chapters covering fruits, seeds, and the dispersal by wind for fruits/seeds in many genera. The chapters on structure–function for legume fruits (Pate) and their seeds (van Staden et al.) illustrate further this diversity and provide reasons for their success. Dispersal by wind (Augspurger) is found in numerous genera in all three subfamilies with diaspores with different morphologies having markedly different aerodynamic properties.

The evolution of genera in the Leguminosae (Small) considers among other topics the ever-present problem faced by biosystematists of what is a genus. "Generiation" in legumes is approached from a variety of perspectives with emphasis on explanation in terms of biological causes and adaptive responses. Useful and harmful legumes are discussed in a pair of interesting chapters covering reproductive changes induced by domestication (Plitmann & Kislev) and the benefits and risks of legume introductions (Hughes & Styles). The results of plants being introduced into exotic habitats has produced both boon and bane. The careful study of the biology of potential introductions is necessary to avoid disastrous consequences when beneficial results are anticipated.

Many leguminous plants are noted for their symbiotic association with *Rhizobium* bacteria and the corresponding ability to produce root nodules and subsequently fix atmospheric nitrogen into compounds useful to the plant. Several papers deal with a range of topics concerning nitrogen fixation and mycorrhizal associations, from the structure and function of root nodules (Sprent et al.), to isotopic differences of nitrogen as a means to study its fixation by legumes (Kohl & Shearer), the interactions of herbaceous legumes with associated rhizobia, mycorrhizae, pests, and parasites (Schenck), the systematics and ecology of ectomycorrhizal legumes (Alexander), and finally, brief reflections on mycorrhizal research carried out over many years in the American tropics (Singer). Genome evolution in legumes (Rees & Narayan) and the coevolution of the genomes of rhizobia and legume host (Verma & Stanley) are given special attention, and future studies in the coevolution of these organisms will surely yield fascinating results.

The lone paper covering legume chemistry concerns cyanogenesis (Seigler et al.) and provides updated information on the known systematic occurrence of cyanogenetic compounds in the family. Another topic of special interest includes the relation between the radiation regime and daily leaf movement in legumes (Herbert). Nyctinastic and helionastic movement in legume species is a focus in our understanding the physiology and ecology for diurnal leaf movement.

Four papers cover interactions between legume species and associated insects such as ants (McKey), bruchid beetles (Johnson and Birch et al.), and aphids (Simmonds et al.) with varying approaches to the subject and the obvious implication that the area of plant–insect interactions is one in great need of close observation and scrutiny by field-oriented researchers. The final paper in this volume (Bisby) addresses databases and information systems and discusses their effect on legume research. The usefulness of such systems is that they can enlarge the range and efficiency of legume

researchers' work by providing modern information support services, thus helping to direct and define interesting and productive projects for investigation.

The meeting in St. Louis and the publication of these proceedings was supported by a grant from the United States National Science Foundation (BSR-8516663); the results of research reported in this volume do not necessarily reflect the views of the Foundation. Other conference-related support was received from the B. A. Krukoff Fund for Legume Research, the General Service Foundation, and the Missouri Botanical Garden. An additional grant from the National Science Foundation International Programs Division (INT-8520861) supported the attendance of ten participants from Latin America, Africa, and Madagascar at the meetings and at a workshop in herbarium management, held at the Missouri Botanical Garden from 16–20 June 1986. Partial travel support allowing several botanical colleagues from the People's Republic of China to participate in the Conference and workshop was received from Mark Twain Bancshares, Inc., St. Louis, through the kind generosity of Mr. Adam Aronson. Support for C. H. Stirton at Kew Gardens was provided by the B. A. Krukoff Fund for Legume Research.

The Conference Organizing Committee wishes to extend thanks to all those who assisted in making the St. Louis meetings a success, with special thanks to Julie Gentry, Jeanne Morgan Zarucchi, and many Garden staff and volunteers who graciously helped the organizers in welcoming and assisting the Conference participants. Additionally, the editors are extremely grateful to numerous colleagues who reviewed manuscripts and provided useful criticism of earlier versions of the papers that appear herein. Finally, we thank George Rogers and editorial assistants Janice Wilson and Amy Scheuler of the Garden's Scientific Publications Department for seeing this work through to printed and bound form.

James L. Zarucchi
December 1989

LIFE HISTORY STUDIES

N. R. Sackville Hamilton[1]

ABSTRACT

A plant grows by the repeated iteration of modular units of construction. The population dynamics of the modules comprising one genet, combined with the morphological constraints on the placement of daughter modules, describe the growth of the genet. These constraints determine plant architecture and, thus, how the plant samples the environment and interacts with other organisms. For example, *Trifolium repens* grows plagiotropically, with all axes indeterminate. Each plant moves through the pasture as new modules are born at the stolon apex and old modules die at its base, leading to low intraclonal interference and a high degree of exploration of new microenvironments. Each new module encounters new neighbors. In one year, one apical meristem may produce 20–40 modules and interfere with 100 neighboring plants. The dynamics of plant populations must be studied at at least two levels in the hierarchy of construction of plants—the genet and the module. A genet of *T. repens* may become separated into physically distinct ramets either by the senescence of connecting modules or by extrinsic damage. Genets, which do not senesce, may become represented by ramets several meters apart, necessitating the use of genetic markers for studies on the population dynamics of genets. Such studies have shown high levels of genetic variation within populations and, despite rare seedling establishment, large numbers of closely intermingled genets rather than a few dominant genets. Mechanisms proposed for the evolution of such population structure include temporarily variable selection pressures, rare flushes of seedlings, local specialization to different neighbors, and the low magnitude of intraspecific relative to interspecific interference.

I. INTRODUCTION

The purpose of this paper is to summarize some of the principles of what has come to be known as the population biology of plants. The plant population biologist adopts an essentially reductionist approach to studying the ecology and evolution of plants. The principles of plant population biology are here illustrated by specific example using *Trifolium repens*. Most of the examples will be taken from a series of studies on a population in a small (1 ha) permanent pasture in North Wales that is believed to be ecologically the most intensively studied ecosystem in the world; descriptions of the field and analyses of its vegetation are given by Sarukhán & Harper (1973), Turkington (1975), Trathan (1983), and Thorhallsdottir (1983).

II. POPULATION BIOLOGY OF PLANTS

Population biology deals with questions about the number of organisms and how it is determined. The number of organisms of a particular species present in an area of land is determined by four processes: 1) the birth rate—the rate at which new individuals are born within the area; 2) the death rate; 3) the immigration rate—the rate at which new individuals move or are dispersed from outside to within the area; and 4) the emigration rate—the rate at which individuals leave the area.

[1]Unit of Plant Population Biology, School of Biological Sciences, University College of North Wales, Bangor, Gwynedd LL57 2UW, United Kingdom.

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The fundamental equation of population biology is therefore:

$$N_{t+1} = N_t + \text{births} - \text{deaths} + \text{immigrants} - \text{emigrants},$$
$$\text{where } N_t = \text{number of individuals at time } t.$$

These are the processes we study in assessing how numbers are controlled. They are affected by the nature of the organisms and of their responses to their environment, and attempts to investigate these effects necessarily have to cover all the classical areas of biology—morphology, physiology, pathology, genetics, and so on. The purpose of this paper is to show how, within the discipline of plant population biology, the architecture and developmental morphology of plants are studied as the basic units for measurement; how these determine the way a plant "samples" its environment, how they influence its response to the biotic and abiotic environment; and how this in turn leads to an analysis of the ecological interactions, the structure and genetics of the population, and the evolutionary responses to selection pressures.

III. The Plant as a Metapopulation

Counting the number of individuals in a population of cows gives a sensible measure of the "amount of cow" present. In contrast, with plants and certain animals such as corals, bryozoans, and colonial ascidians, deciding what to count presents a major problem. For example, a population of *Pteridium aquilinum* might contain two individuals, one occupying 1,500 m^2 of land (Oinonen, 1967) and the other just 0.01 m^2. The statement that there are two individuals in the population is not an adequate summary of how much bracken is present. This problem has been the primary factor limiting the past development of a science of plant population biology. The problem exists because such organisms grow by the repeated iteration of modular units of construction, or *modules*.

The term module has been variously defined in the literature. As used here, "the module is the multicellular unit of structure that is iterated in the process of growth" (Harper et al., 1986). Many authors prefer a more precise, morphological definition (e.g., Barthelemy, 1986; Hallé et al., 1978; Hallé, 1986; White, 1984). However, such definitions inevitably lead to a loss of generality, because there is no homology of architecture common to the diverse groups of organisms that show this iterative growth form. We therefore use the more general definition, allowing more precise, species-specific, morphological definitions as required.

The metapopulation (White, 1984) of modules derived by mitotic cell division from one zygote is termed the *genet*. The growth of genets can be described in terms of the population dynamics of their modules. If a genet has n_t modules at time t, then the number present at time $t + 1$ is

$$n_{t+1} = n_t + \text{births} - \text{deaths},$$

and the total number of modules in the population is the sum of the number of modules in each genet. Of course, it is not adequate to treat the modules merely as a population of unrelated individuals; it is essential to analyze their dynamics in the context of the developmental morphology, or architecture, of the genet, since this determines how and where they are born and die.

The genet is the individual of evolutionary interest, so the population biologist needs to count genets. However, the module is the unit for acquisition of resources and growth, so the module population determines productivity and fitness of genets. Therefore, the population of modules must also be counted.

The important point is that a plant population is in reality a population of populations. Classical theory for population dynamics, population genetics, and evolution based on nonmodular organisms is inappropriate. A fundamentally different theory is being developed (e.g., Taylor & Williams, 1984; Sackville Hamilton et al., 1987) in which, for example, the size of a genet has a major effect on its fitness. In contrast to unitary organisms, there is found to be no theoretical reason to expect

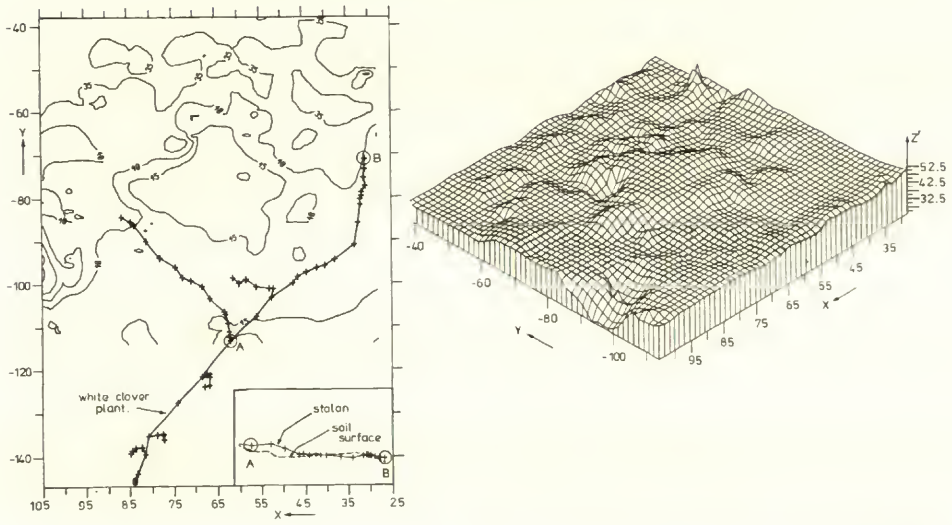

FIGURE 1. Contour diagram (left) of the surface of a 180 × 130 cm patch of soil and the corresponding surface trace (right), obtained with a three-dimensional digitizer in the field. A trace of the stems of one plant of *Trifolium repens* is superimposed on the contour diagram (+ = node of a module; ● = senescing base of the plant) with (inset) a vertical section through part of the stem and the soil surface in the same vertical plane. Dimensions are in millimeters. From Jones et al. (1985), reprinted with permission from The Institute of Measurement and Control, London.

the evolution of senescence of genets, a result supported by empirical evidence for the absence of senescence in certain types of clonal organisms (Bell, 1984; Buss, 1985; Comfort, 1979).

To count modules and estimate rates of birth and death, it is necessary to mark individual modules, relocate them at frequent intervals, and thus determine their fate. However, they must be marked without damage, using markers that are invisible to sheep, birds, and other potentially inquisitive animals whose effect on the plants would be modified if they could see the marks. Following populations in this kind of detail can be extremely laborious, although the rate of data collection can be increased by the use of data loggers. Jones et al. (1985) described an instrument used to construct repeated three-dimensional maps of the same 1 × 1 × 0.5 m plot within a pasture to an accuracy, repeatability, and resolution of better than 1 mm. Figure 1 shows an example of a reconstruction of the soil surface and of two connected shoot axes of *Trifolium repens* growing over it, which was obtained with the instrument.

IV. Plant Architecture and Dynamic Morphology

In *Trifolium repens*, as in many other plants, there are several levels in a hierarchy of organization (Table 1). Under the general definition of module, individuals at any level below the genet could be described as modules, although for the purposes of this paper it is convenient to use the term module to refer to the node with its associated internode, axillary bud, petiole, and leaf.

Table 2 summarizes the architectural constraints governing the growth of genets of *Trifolium repens*. Many of the characteristics of populations of *T. repens* are directly attributable to these constraints. For example:

TABLE 1. Descriptions of individual units at various levels in the hierarchy of organization of *Trifolium repens*.

Individual	Description
Module	A node on a horizontal stem, together with its associated internode, axillary bud, petiole, and leaf.
Apical meristem	A meristem at the apex of a shoot axis, with cells dividing and differentiating modules. An axillary bud becomes an apical meristem as soon as it becomes active and vegetative.
Shoot axis	A linearly connected set of modules, differentiated sequentially by one apical meristem at its apex.
Ramet	A set of branching shoot axes that is readily perceived as an "individual plant" in the field.
Physical individual	A physically, not necessarily physiologically, connected set of ramets. Identification generally involves destructively tracing the component shoot axes over and through the soil.
Genet	The population of modules derived by clonal growth from one zygote: "the genetic individual." Comprises one or more ramets and physical individuals. Identification of mature genets is possible only by using genetic markers.

TABLE 2. Architectural rules governing the growth of adult plants of *Trifolium repens*.

Stems grow horizontally over the soil surface.
Phyllotaxy alternate, distichous.
Growth indeterminate.
Single axillary bud at each node. Probability of developing varies between 0 and 1.
Axillary bud may develop into an inflorescence or into a branch.
All internodes elongated. Internode length variable from 0.1 cm to 5 cm.
Leaves senesce. Senesce after 1–3 months.
Modules senesce. Senesce after 1–2 years.
Apical meristems do not senesce.
One, occasionally two, adventitious roots may develop at each node, sufficiently well to support the requirements of the whole plant. Probability of developing varies between 0 and 1.

1. Because its stems are all horizontal with elongated internodes, the growing points continually move laterally through the sward.
2. Because the apical meristem neither senesces nor loses its meristematic activity, and because the adventitious roots develop sufficiently well to support the plant independently of the original seedling tap root, the shoot apex, and thus also the genet, is potentially immortal.
3. Because its modules senesce, the genet divides into an unlimited number of physically separate plants as it grows.
4. Each physically separate plant effectively moves laterally through the sward as it grows; the growing points move forward as new modules are born, while the base retreats as old modules senesce.
5. Because the probability of a branch developing from an axillary bud varies from 0 to 1, the plant can grow as anything from one unbranched stem to a dense, highly branched structure.
6. Because a leaf senesces before the rest of its module, only the youngest modules on a shoot axis have living leaves. Thus the light-gathering part of the plant is largely restricted to the apical portion of the stem.
7. Because each adventitious root develops relatively late in the module's life and may live as long as the node from which it develops, only the older modules have established roots, and the nutrient-gathering part of the plant is restricted to the basal portion of the shoot axis.

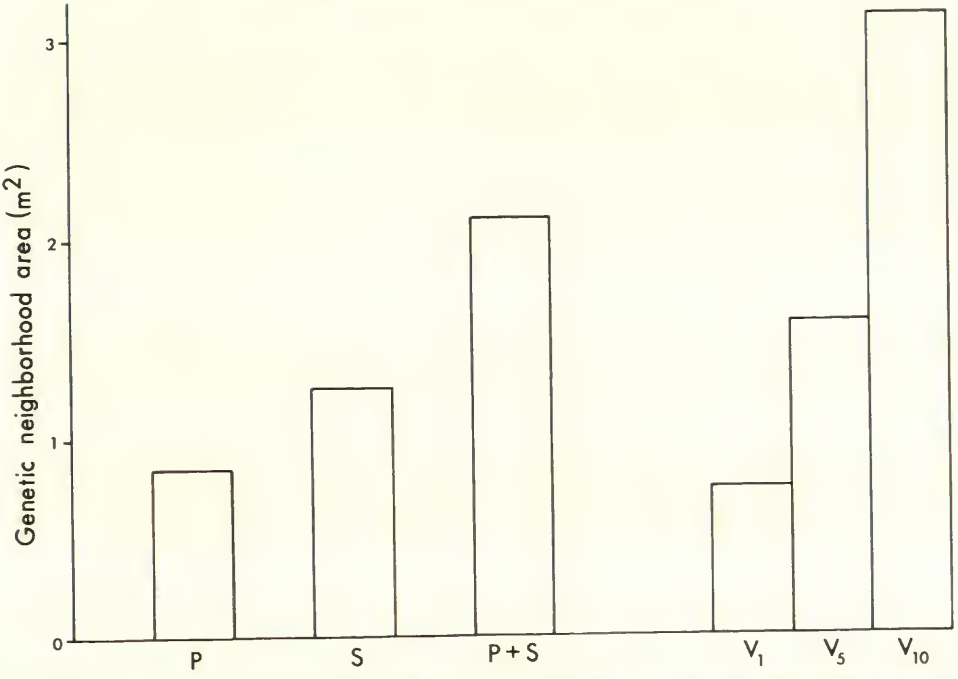

FIGURE 2. Components of the estimated genetic neighborhood area of *Trifolium repens* attributable to pollen dispersal (P), seed dispersal (S), and to vegetative growth for i years (V_i). From Gliddon & Saleem (1985).

V. Implications for Gene Flow

These architectural constraints influence the rate at which genes can be dispersed through a population and, therefore, the rate at which the population can respond evolutionarily to selection. In plants, gene dispersal is usually considered to occur partly by pollen dispersal and partly by seed dispersal, but horizontal growth also contributes to gene flow by causing flowers to be placed at a distance from the site at which the genet originated as a seedling.

The probable distance between a genet and its parents is limited by gene dispersal. Within a certain distance of each plant, gene dispersal is sufficiently high to permit the assumption that any of the older plants within that region are equally likely to have been its parents. The region within that distance is the *genetic neighborhood* (Wright, 1943, 1946, 1969, 1978). It is a measure of the effective genetic population size and is calculated using estimates of gene dispersal variance.

Gliddon & Saleem (1985) estimated the three components of the area of the genetic neighborhood of *Trifolium repens* (Fig. 2). They estimated pollen dispersal by measuring the distances traveled by pollinating insects from one flower to the next; seed dispersal by measuring the distribution of seedlings establishing in sterilized soil around isolated mother plants; and "vegetative dispersal" by using the architectural rules for growth of *T. repens*, with parameters estimated from various experimental observations, as input for a general computerized simulation of plant growth (A. D. Bell, 1985). Gene dispersal attributable to horizontal extension increases as the plant grows. After five years of growth it was the largest single component of gene flow, and after ten years it accounted for 60% of all gene flow. The proportion actually contributed by growth in an established population will depend on the age and size structure of the population of genets, which is impossible to measure directly because of module senescence. Suffice it to observe that there is no theoretical

upper limit to the age of the genets, and the only known estimate of longevity of genets of *T. repens* in natural populations (Harberd, 1963) was up to 100 years. The extent to which horizontal extension contributes to the rate of gene dispersal itself, rather than to the size of the genetic neighborhood, depends in part on V_1, the annual dispersal rate, which is similar in magnitude to pollen and seed dispersal.

VI. Implications for Environmental Sampling

Architectural constraints also influence the way in which *Trifolium repens* encounters fine-scale environmental heterogeneity. Permanent pastures are highly heterogeneous at the scale of individual modules of *T. repens* in terms of the local distribution of species, patches of bare soil, dung and urine, molehills, wormcasts, trampling, grazing by large and small animals, infection by pathogens and pests, rhizoflora and fauna, soil fertility, etc. Each new module produced by an apical meristem effectively encounters a new environment. In one study in north Wales (Sackville Hamilton & Harper, 1989), it was estimated that an apical meristem may produce 10–50 modules and encounter up to 100 neighboring plants during the course of one year.

Such spatial heterogeneity is encountered by *Trifolium repens* as temporal heterogeneity, patches being exploited first by the leaves and subsequently by the roots as the shoot apex enters and grows through the patch. It may be encountered as coarse-grained (Levins, 1968) heterogeneity at the level of the shoot axis and genet, but fine-grained at the level of the module. At any one time, the neighbors with which the shoot axis competes for light are not the neighbors with which it competes for nutrients.

VII. Integrated Dynamics

To understand the importance of these observations, it is necessary to study the responses of the plant to its environments in terms of the rates and distribution of births and deaths of modules and of genets. However, the processes of births, deaths, and growth of individuals at the various levels of organization are not mutually independent. Table 3 describes the processes at each level in terms of what processes they represent at other levels. A branch is born when it first becomes visible in the axil of a leaf, after the axillary bud becomes active and vegetative; this is the same as "birth" of an apical meristem. A module is born when its leaf appears at the stem apex, after differentiation of cells in the apical meristem. Finally, birth of the genet involves the appearance of the seedling above ground. Note the distinction between conception (e.g., fertilization of the zygote) and birth, when the new individual first becomes visible.

Death affects only apical meristems and modules directly, shoot axes and genets dying only as a result of the death of all their component modules. Growth of a shoot axis involves the birth, death, and growth of its component modules. Movement of a shoot axis involves movement of its apex and senescence of its oldest modules; movement of the apex itself occurs as a result of the production and elongation of modules by the apex.

Because of these interrelationships, the causes of births and deaths can be fundamentally different for each level (Table 4). Birth of leaves and modules is controlled intrinsically by the ontogeny of meristem growth, and birth of branches may be controlled by apical dominance (but see the experiment on apical dominance described below). The senescence of modules is an intrinsic cause of genets splitting into physically separate plants as they grow. Birth of genets is under intrinsic control of a complex set of factors commonly referred to as the *reproductive strategy*, determining the timing, nature, and extent of investment in sexual reproduction. Death is caused intrinsically by senescence. However, as noted earlier, this occurs only at the level of leaves and modules. There is no intrinsic control of mortality of apical meristems, nor, therefore, of branches and genets.

TABLE 3. Interrelationships among individuals at different levels of organization in *Trifolium repens* expressed in terms of their births, deaths, growth, and movement.

	Process or event			
Individual	Birth	Death	Growth	Movement
Apical meristem	Birth of shoot axis			Production and extension of modules
Module	Appearance of leaf at the apex of a shoot axis			
Shoot axis (branch)	Appearance of branch in axil of leaf	Death of all component modules	Birth, death, growth of component modules	Movement of apex and death of old modules
Physical individual	Death/severance of connecting modules	Death of all component shoot axes	Birth, death, growth of component shoot axes	Birth, death, growth, movement of component shoot axes
Genet	Appearance of seedling above ground	Death of all component ramets	Birth, death, growth of component ramets	Birth, death, growth, movement of component ramets

TABLE 4. Factors directly responsible for births and deaths of individual at each level in the organization of *Trifolium repens*.

	Control of Births		Causes of Death	
Individual	Intrinsic	Extrinsic	Intrinsic (Senescence)	Extrinsic
Leaf	Ontogeny fixed		Senesces after 1–3 months	Grazing, pathogens, climate, trampling
Apical meristem	Apical dominance?	Neighbors, local light, temperature, resource availability	No senescence	Grazing, pathogens, climate, trampling, burial, death of supporting modules
Module	Ontogeny fixed	Resource availability, interference	Senesces after 1–2 years	Grazing, pathogens, climate, burial, death of supporting modules
Shoot axis	As for apical meristem	As for apical meristem	None	Systemic pathogens
Ramet	Senescence of modules	Severance of modules	None	Systemic pathogens, severance of all roots
Genet	Reproductive strategy—flowering, seed set, dormancy, dispersal	Factors affecting flowering, fertilization, abortion, dormancy, seed mortality, germination	None	None

TABLE 5. Summary of attributes of the growth of *Trifolium repens* that influence its tendencies to explore new territory and to fill occupied territory.

Tendency to explore new territory = mean length of internodes on a shoot axis × rate of production of new modules by apical meristem
Tendency to fill occupied territory increases with:
increasing probability of branching
decreasing age of module at branch initiation
increasing rate of production of new modules by apical meristem
decreasing length of internodes
increasing size of leaves
increasing longevity of leaves
increasing probability of root initiation
decreasing age of module at root initiation
increasing size of roots

Similarly, although there is a wide set of environmental factors affecting births and deaths, none of these are directly responsible for the deaths of whole genets—only indirectly, by causing the death of all their modules. At the level of the ramet, only systemic pathogens can be directly responsible for mortality. Thus, when studying the mortality of genets, it is only meaningful to treat it as the result of mortality of all its modules.

Different attributes of the life history of modules contribute in different ways to the control of the rate and distribution of births and deaths. Lovett Doust (1981) coined the terms "phalanx" and "guerrilla," by analogy with contrasting strategies of warfare, to refer to two extremes in a continuum of module life histories. A plant with phalangeal growth places each new module close to its parent module, resulting in a tightly packed genet that is slow to spread but resistant to invasion by other plants. At the other extreme, the guerrilla plant, such as *Trifolium repens*, places daughter modules far from their parent modules, resulting in a rapidly moving genet with widely spaced modules easily invaded by other plants. This single continuum has two correlated facets: a tendency to explore new territory, high in the guerrilla plant; and a tendency to fill occupied territory, high in the phalangeal plant. Table 5 summarizes the parameters of the life histories of modules of *T. repens* that influence these tendencies and, thus, the parameters of interest to the population biologist.

VIII. Studies on Metapopulations of Modules

The length of the internode is the critical feature distinguishing growth habits along the phalanx–guerrilla continuum—and affects both the tendency to explore and the tendency to occupy. It has been shown to vary plastically in response to changes in the environment, such as neighbors, light, and soil conditions, as well as to different types of physical obstruction such as matchsticks stuck in the soil. There is also considerable genetic variation, both within and between populations in this character and in its response to environment. Burdon (1980), Sackville Hamilton (1980), and Solangaarachchi (1985) present some examples of these effects. It thus provides one way in which genets of *Trifolium repens* can respond to their environment.

The other component of the tendency to explore—the rate of module production by a single apical meristem, which is also one determinant of the rate of birth of modules—also shows genetic variation within and between populations but less phenotypic plasticity to environmental factors other than seasonal changes (Harvey, 1979; Sackville Hamilton, 1980; Sackville Hamilton & Harper, 1989). Thus, it appears to provide relatively little scope for responding to spatial heterogeneity of the environment.

Of the parameters that affect only the tendency to occupy a patch of ground, the mean size of

leaves and the longevity of leaves vary both genetically and plastically. Genetic variation in the former has, in conjunction with correlated characters, a major influence on the type of environment in which the plant grows best—so much so that it is used as the principal variable in classifying commercially available cultivars. By virtue of the unitary growth form of leaves, however, both can necessarily have only a limited influence on the tendency of the plant to occupy territory. In contrast, changing the probability and rate of branch initiation can have a much greater effect. Indeed, plastic variation in the branching of *Trifolium repens* is such that it can become a typical phalanx plant, at least in terms of the occupation of territory, though not of exploration of new territory; a single clone may occupy an area of land to the virtual exclusion of other plants of the same and of other species (Sackville Hamilton, 1980). In the absence of sexual reproduction, initiation of a branch is the only way in which the number of growing points can increase. It is thus the most important variable controlling the birth rate of modules.

There have been numerous studies on the control of branching in *Trifolium repens,* all of which can be interpreted in terms of births of modules. Genetic variation within and between populations, both in branching and its response to environment, has been demonstrated by Sackville Hamilton (1980), for example. Harvey (1979), Solangaarachchi (1985), and Newton (1986) review some of the factors controlling branching in the species. The factors studied include temperature, intensity, and quality of light; nutrient supply; interference with neighboring plants; defoliation; physical barriers to plant growth; and the internal regulation of branching by apical dominance, or correlative inhibition of dormant lateral buds by active apical meristems.

If *Trifolium repens* can exploit locally favorable microenvironments as it grows through patches in a heterogeneous pasture, then its individual modules must be able to respond independently to their immediate environment. It is therefore necessary to determine the extent to which individual modules can respond independently, as against the extent to which there is physiological control of branching, by correlative inhibition.

Correlative inhibition, or apical dominance, has often been demonstrated in laboratory conditions but less often in the field. The laboratory studies have been carried out under highly favorable conditions, where the factors limiting the rate of growth may be quite different from those limiting growth in the field. Under optimal environmental conditions, plant growth is more likely to be limited by the plant's own physiological constraints than where the environmental factors are themselves severely limiting growth. Bülow-Olsen et al. (1984) observed that at the border between two abutting genets of *Trifolium repens* the density of apices was no higher than away from the border, suggesting that the production of apices responded to the vicinity of other apices regardless of whether or not they were connected, and thus providing circumstantial support for the hypothesis that local environmental conditions are more important than intrinsic correlative inhibition in the control of branching.

On the other hand, Sackville Hamilton & Harper (1989) have observed what might be attributable to apical dominance in the field. They marked plants of *Trifolium repens* in the field, traced their growth for a period of one year, and found that 39% of the stems were decapitated by grazing or trampling. In every case decapitation of the stem was followed by the rapid development of a branch, either of a preexisting branch or, if no branches existed before decapitation, of a new branch from the axillary bud of the youngest module basipetal to the cut.

To test the hypothesis experimentally, Solangaarachchi (1985) repeated the classical test for correlative inhibition (removal of the apex) with two critical differences. First, the stem was cut at the base of a fully developed sward, with limited light and nutrients for growth. Second, the stem acropetal to the cut, which is normally discarded, was left to continue growing. This is possible in *Trifolium repens* because of the development of adventitious roots at the nodes. She observed increased branching basipetal to the cut, in agreement with classical results (Fig. 3). However, she also observed increased branching acropetal to the cut in those nodes still connected to the apical meristems. If the former phenomenon is considered evidence for apical dominance, then the latter is evidence for basal dominance.

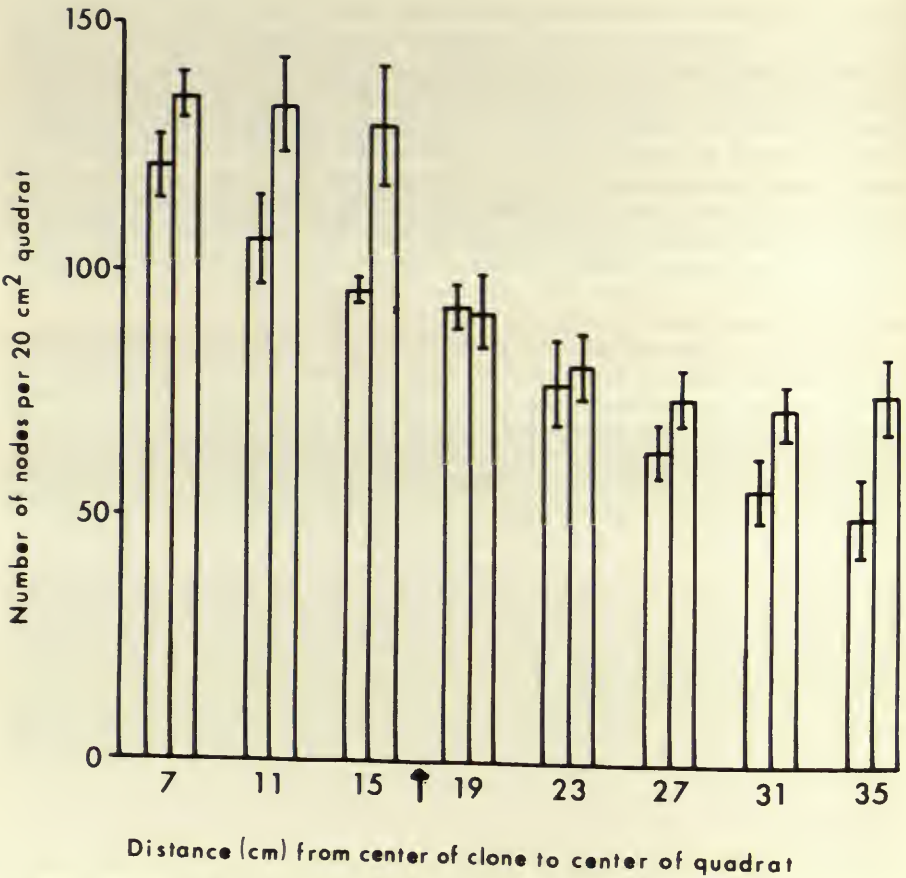

FIGURE 3. Variation in the density of nodes from the center to the edge of isolated clones of *Trifolium repens*, measured as the number of nodes in each of eight contiguous 5 × 4 cm quadrats. Two bars are drawn for each quadrat position: the left in each pair for the control, the right for clones severed with a circular cut through all stolons at the point marked by the vertical arrow, 17 cm from the center of the clone. 95% confidence intervals are shown for each bar. Redrawn from Solangaarachchi (1985).

There are two possible explanations for the response of a bud to cutting a stem connected it. First, the stem may have been the path by which an inhibitory substance is normally translocated to the bud and cutting the stem prevents that transport—the classical story. Second, cutting the stem may cause the secretion of a stimulatory substance at the cut that is not normally present—a wound response that plays no part in the normal regulation of branching other than stimulating the replacement of lost apical meristems. The known stimulatory effect of defoliation on the development of axillary buds (e.g., Newton, 1986) could equally be interpreted as a wound response.

Newton (1986) performed an elegant series of experiments on the local branching responses of modules. He cut segments of a runner containing precisely three modules, each with a leaf but no branch, and applied different treatments to each module. He applied different levels of nutrients, intensities of light, and amounts of defoliation to the three connected modules. Initiation of a branch on a module was delayed by low levels of nutrients and of light applied just to that module and was stimulated by removal of the leaf subtending that module. Modules adjacent to the treated module

were affected either less or not at all. For example, changing nutrient levels supplied to one module also affected branching at the adjacent acropetal module, although to a lesser extent than at the treated module, whereas the basipetal module was unaffected. There was some evidence that both acropetal and basipetal modules were affected by shading and defoliating one module. Newton's experiment thus clearly shows the ability of the plant to respond extremely locally to its microenvironment, each module showing a specific local response.

IX. Studies on Populations of Genets

The above shows that genets of *Trifolium repens* are highly mobile, potentially immortal, and able to respond locally to changing conditions, thus exploiting locally favorable microenvironments and passing through unfavorable ones. The studies described dealt with genets as populations of modules but did not deal with the population of genets.

Because genets fragment into distinct ramets as they grow, it is technically impossible to determine the ages or rates of mortality of genets. Even counting and identifying genets is impossible without recourse to genetic markers. Polymorphisms that have been used as markers in *Trifolium repens* include leaf marks, cyanogenesis, the *s*-alleles determining self-incompatibility, and electrophoretically characterized isozymes (Cahn & Harper, 1976; Sackville Hamilton, 1980; Trathan, 1983).

Cahn & Harper (1976), identifying phenotypes by their leaf marks, demonstrated close intermingling of large numbers of genets—up to ten in a 100 cm^2 area. Sackville Hamilton (1980), using a combination of leaf marks, cyanogenic phenotype, the *s*-alleles determining incompatibility, and other morphological characteristics to identify genets, found similar extremely close intermingling of genets in other, similar fields, although fewer or younger genets elsewhere.

Trathan (1983) sampled 50 ramets from each of seven 1 m^2 plots and used electrophoresis to identify the genotype at 21 loci, thus classifying the sampled ramets into genets. He then used these data to attempt to determine the total number of genets present in the field by plotting the number of genets isolated against the number of ramets sampled. Theoretically, the slope of the graph should decrease as the number of ramets sampled increases, tending asymptotically to the total number of genets in the population. However, the increase was linear (Fig. 4). He isolated 47–50 new genets from each new set of 50 ramets sampled, with no indication of an approach to an asymptote. He therefore concluded that there are at least 47 distinct genets per m^2, the qualifier "distinct" being used to mean that each square meter contains 47 genets not already counted in other square meters. The actual density of genets must be considerably higher, because the density of ramets was much higher than 50 per m^2; and isolating up to 50 genets from 50 ramets suggests that there were other genets to be found among the unsampled ramets.

The birth of new genets in the population has only rarely been observed, although Jayasingham (1985) found an average of 140 seeds per m^2 in the seed bank. In the east of England, Sackville Hamilton (1980) found that the establishment of seedlings of *Trifolium repens* was common in some populations but not observable in others. In the field of north Wales, it appears to be so rare that quantitative estimates of birth rates have been impossible, most workers having seen just one or two seedlings during the course of long, intensive experiments in the field (e.g., Turkington, 1975; Thorhallsdottir, 1983), and then mainly in gaps such as molehills.

The failure to observe seedling establishment may not mean that seedlings are not establishing. For example, there may occasionally be years in which the particular combination of conditions is conducive to abundant seedling establishment, with little or no germination occurring in most years. Any attempt to obtain a reliable estimate of the birth rate of genets would then have to cover many years to be certain of including, by chance, some of the years of abundant germination. Alternatively, if most new genets become established in gaps, while most workers study the fully

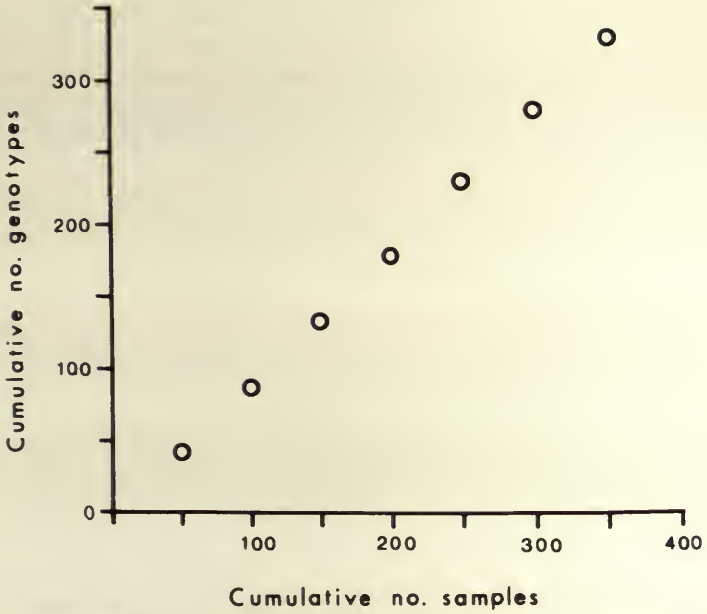

FIGURE 4. Cumulative number of electophoretically characterized genotypes isolated from a population of *Trifolium repens*, against the number of cuttings sampled from the population. Redrawn from Trathan (1983).

developed sward away from those gaps, then the establishment of seedlings will be occurring without being observed. However, one attempt to study recolonization of gaps failed to reveal significant establishment of seedlings of *Trifolium repens* (L. Assemat, unpublished data). Nevertheless, it is safe to conclude that the birth rate of new genets is very low, at least within the sward and in most years.

The rate of mortality of genets cannot be estimated. To record the death of a genet, it would be necessary to know the location of all its component modules while they were still alive and then follow that population of modules to extinction. Because genets split into physically separate parts as they grow, there is no known way of doing this; the process of locating and identifying all modules would involve destroying the whole population. It may be assumed, however, that the population is stable, at least in the long term, and therefore that the rate of death equals the rate of birth—that is, the death of genets must be as rare as their birth.

Thus, over the whole field, the high numbers of genets are maintained in a state of low flux, even though each genet comprises a mobile population of modules in a state of high flux. The average age of genets must perforce be high. If 1) the birth rate of genets is assumed to be in the region of one genet per m^2 per year (rather a wild estimate based on incidental observations); 2) the overall number of genets is constant; and 3) the overall density of genets is at least 47 distinct genets per m^2 (Trathan, 1983), and almost certainly much higher; then the average age of genets must be at least 47 years.

This state of low flux applies only over the whole field. At the scale of the effective genetic population size—i.e., around 5 m^2 (Gliddon & Saleem, 1985)—the high rates of birth, death, and dispersal of modules of each genet causes the genets to be highly mobile. Thus, the rates of immigration and emigration of genets into and out of effective genetic populations are high, resulting in a high flux in the number of genets despite low rates of birth and death.

X. Maintenance of Number and Diversity of Genets

The above studies have demonstrated the maintenance of large numbers of genets within one population. In addition, Burdon (1980) has demonstrated that these genets are highly diverse genetically. Comparative studies (e.g., Sackville Hamilton, 1980; Turkington, 1985) have shown that the results for this one population are typical also of other populations of the species.

The potential immortality of genets and their ability to spread throughout the field by clonal growth might lead one to expect the population to be dominated by just the few fittest genets, contrary to actual findings. It is therefore necessary to explain how such a large number of such diverse genets can coexist in the one population.

Burdon (1980) suggested that the diversity resulted from biotic selection pressures, such as pathogens, that were variable within the field. Several studies have supported this suggestion. For example, the population is polymorphic for cyanogenesis, cyanogenic phenotypes being those that release cyanide when damaged, thereby gaining partial protection from grazing by mollusks. Dirzo & Harper (1982) demonstrated that the grazing pressure from mollusks varied within the field and that the distribution of cyanogenesis in the population of *Trifolium repens* was correlated with the distribution of mollusks. Cyanogenic phenotypes were more abundant where mollusks were more abundant, thus providing a clear example of local differentiation within the population in response to local variation in selection pressures.

Turkington (1985) has shown local differentiation of the population with respect to neighboring grass, whereby a plant of *Trifolium repens* sampled from beside *Lolium perenne*, for example, grew better in competition with *Lolium perenne* than with *Cynosurus cristatus*, and vice versa for a plant sampled from beside *Cynosurus cristatus*. Trathan (1983) and Gliddon & Trathan (1985) have similar evidence of even finer local differentiation with respect to the neighboring genet of *Lolium perenne*. Turkington (1975) and Trathan (1983) have also found local specialization with respect to unspecified sources of heterogeneity within the field. Plants of *T. repens* taken from the field and transplanted back into several different sites within the field grew and survived better when transplanted back into the site from which they were originally taken than when transplanted back into sites from which other ramets of *T. repens* were taken. There is thus clearly local specialization of genets within the population. This may be the result of evolution and subdivision of the population involving differential natality and mortality of genets, but not necessarily so. The population can also become locally specialized as a result of the lateral movement of genets by clonal growth and consequent redistribution of genets throughout the field, with no change in the overall population of genets.

It is also possible that the *Trifolium repens* is not itself the active agent of specialization. For example, different races of *Rhizobium* or other microbes in the rhizosphere may show different responses to different genets of *T. repens*. There would then develop differences in the rhizosphere correlated with the distributions of genets of *T. repens*. The apparent specialization of *T. repens* would be the result of specialization of the microfauna and microflora to *T. repens*.

There is also the possibility that *Trifolium repens* may be subject to somatic selection in the broad sense of environmentally induced phenotypic changes that are stable over a period of a year or more, not necessarily genetic or heritable through the zygote. Such changes have been observed, for example, by Breese et al. (1965) in perennial ryegrass. If this were the case, then the assumption that genets can be distinguished by their *s*-alleles or electrophoretic isozymes might not be valid. Then the results described here would not necessarily demonstrate the close intermingling of large numbers of genets. Rather, they might possibly be attributable to fine-scale somatic selection, howsoever caused. There is a lack of direct evidence for such somatic selection, although the hypothesis is being tested in other populations of *T. repens* (R. Turkington, pers. comm.).

Although the above work demonstrates that local specialization accounts at least in part for the genetic diversity found within the population, it does not discount other possible explanations. In particular, temporally changing selection pressures—in which, for example, there might be strong

selection for rust one year, strong selection for drought tolerance the next—might also be a contributing factor, although difficult to estimate by virtue of its unpredictability from year to year.

A further mechanism has been proposed by Sackville Hamilton & Harvey (in prep.) based on comparisons with natural, mature populations that do not have large numbers of genets. These populations are characterized by dominance of *Trifolium repens* to the near exclusion of other species. In addition, those few dominant genets are clearly separated in their distribution within the population, in contrast to the close intermingling found in most populations. This is easily explained on the basis of competitive exclusion occurring between genets, resulting in patches dominated by single genets.

Those populations that comprise many closely intermingled genets of *Trifolium repens* are not the dominant component of the sward. Individual modules in such populations therefore interfere mainly with modules of other species (interspecific interference) rather than with other modules of the same species (intraspecific interference). Competitive exclusion of one genet by another results from intraspecific interference. Therefore, where interspecific interference is more important than intraspecific interference in determining the growth and survival of genets, competitive exclusion of one genet by another is not expected to occur. This hypothesis would help to explain the very close intermingling of diverse genets in a way that local specialization of genets cannot.

XI. Conclusions

In this paper, the principles of the population biology of plants have been illustrated by reference to *Trifolium repens*. Although the particular results described apply only to this species, the methodology and philosophy are applicable to any clonal organism, be it plant or animal, legume or not. It has been shown how new theories are needed to deal adequately with the population biology of clonal organisms and how an understanding of their ecological interactions and evolutionary responses requires the integrated application of diverse branches of biology.

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References

Barthelemy, D. 1986. Establishment of modular growth in a tropical tree: *Isertia coccinea* Vahl. (Rubiaceae). *In* J. L. Harper, B. R. Rosen & J. White (editors), Growth and Form in Modular Organisms. Philos. Trans., Ser. B 313: 89–94.

Bell, A. D. 1985. On the astogeny of six-cornered clones: an aspect of modular construction. Pp. 187–207 *in* J. White (editor), Studies on Plant Demography: A Festschrift for John L. Harper. Academic Press, London.

Bell, G. 1984. Evolutionary and nonevolutionary theories of senescence. Amer. Naturalist 124: 600–603.

Breese, E. L., M. D. Hayward & A. C. Thomas. 1965. Somatic selection in perennial rye grass. Heredity 20: 367–379.

Bülow-Olsen, A., N. R. Sackville Hamilton & M. J. Hutchings. 1984. A study of growth form in genets of *Trifolium repens* L. as affected by intra- and interplant contacts. Oecologia 61: 363–387.

Burdon J. J. 1980. Intra-specific diversity in a natural population of *Trifolium repens*. J. Ecol. 68: 717–735.

Buss, L. W. 1985. The uniqueness of the individual revisited. Pp. 467–505 *in* J. B. C. Jackson, L. W. Buss & R. E. Cook (editors), Population Biology and Evolution of Clonal Organisms. Yale Univ. Press, New Haven, Connecticut.

CAHN, M. A. & J. L. HARPER. 1976. The biology of the leaf mark polymorphism in *Trifolium repens* L. 1: Distribution of phenotypes at a local scale. Heredity 37: 309–325.

COMFORT, A. 1979. The Biology of Senescence, 3rd edition. Elsevier, New York.

DIRZO, R. & J. L. HARPER. 1982. Experimental studies on slug–plant interactions. IV. The performance of cyanogenic and acyanogenic morphs of *Trifolium repens* in the field. J. Ecol. 70: 119–138.

GLIDDON, C. & M. SALEEM. 1985. Gene flow in *Trifolium repens:* an expanding genetic neighbourhood. *In* P. Jacquard, G. Heim & J. Antonovics (editors), Genetic Differentiation and Dispersal in Plants. NATO ASI Series G: Ecological Sciences, 5: 299–319. Springer-Verlag, Berlin.

_____ & P. TRATHAN. 1985. Interactions between white clover and perennial ryegrass in an old permanent pasture. Pp. 161–169 *in* J. Haeck & J. W. Woldendorp (editors), Structure and Functioning of Plant Populations, 2. North-Holland, Amsterdam.

HALLÉ, F. 1986. Modular growth in seed plants. *In* J. L. Harper, B. R. Rosen & J. White (editors), Growth and Form in Modular Organisms. Philos. Trans., Ser. B 313: 77–87.

_____, R. A. A. OLDEMAN & P. B. TOMLINSON. 1978. Tropical Trees and Forests: an architectural analysis. Springer-Verlag, Berlin.

HARBERD, D. J. 1963. Observations on natural clones of *Trifolium repens* L. New Phytol. 62: 198–204.

HARPER, J. L., B. R. ROSEN & J. WHITE. 1986. Preface. *In* J. L. Harper, B. R. Rosen & J. White (editors), Growth and Form in Modular Organisms. Philos. Trans., Ser. B 313: 3–5.

HARVEY, H. J. 1979. The Regulation of Vegetative Reproduction. Ph.D. thesis. University of Wales, Cardiff.

JAYASINGHAM T. 1985. Comparative Demography of Species of Compositae in a Permanent Pasture. Ph.D. thesis. University of Wales, Cardiff.

JONES, D. I., D. J. STEDHAM & N. R. SACKVILLE HAMILTON. 1985. Measurement of plant growth in three dimensions. Trans. Inst. Measurement & Control 7: 145–150.

LEVINS, R. 1968. Evolution in Changing Environments: some theoretical explorations. Princeton Univ. Press, Princeton, New Jersey.

LOVETT DOUST, L. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). 1. The dynamics of ramets in contrasting habitats. J. Ecol. 69: 743–755.

MACKIE, G. O. 1986. From aggregates to integrates: physiological aspects of modularity in clonal animals. *In* J. L. Harper, B. R. Rosen & J. White (editors), Growth and Form in Modular Organisms. Philos. Trans., Ser. B 313: 175–196.

NEWTON, P. C. D. 1986. The Establishment, Growth and Fate of White Clover Plants, with Special Reference to the Physiology of Stolon Growth. Ph.D. thesis. University of Wales, Cardiff.

OINONEN, E. 1967. The correlation between the size of Finnish bracken (*Pteridium aquilinum* (L.) Kuhn) clones and certain periods of site history. *Acta Forest. Fenn.* 83: 1–51.

SACKVILLE HAMILTON, N. R. 1980 [1982]. Variation and Adaptation in Wild Populations of White Clover (*Trifolium repens* L.) in East Anglia. Ph.D. thesis. University of Cambridge. University Microfilms International, Ann Arbor, Michigan.

_____ & J. L. Harper. The dynamics of *Trifolium repens* in a permanent pasture. 1. The population dynamics of leaves and nodes per shoot axis. Proc. Roy. Soc. London, Ser. B, Biol. Sci. (in press).

_____, B. SCHMID & J. L. HARPER. 1987. Life-history concepts and the population biology of clonal organisms. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 232: 35–37.

SARUKHÁN, J. & J. L. HARPER. 1973. Studies on plant demography: *Ranunculus repens* L., *R. bulbosus* L. and *R. acris* L.: I. Population flux and survivorship. J. Ecol. 61: 675–716.

SOLANGAARACHCHI, S. M. 1985. The nature and control of branching pattern in white clover (*Trifolium repens* L.). Ph.D. thesis. University of Wales, Cardiff.

TAYLOR, P. D. & G. C. WILLIAMS. 1984. Demographic parameters at evolutionary equilibrium. Canad. J. Zool. 61: 2264–2271.

THORHALLSDOTTIR, T. E. 1983. The Dynamics of a Grassland Community with Special Reference to Five Grasses and White Clover. Ph.D. thesis. University of Wales, Cardiff.

TRATHAN, P. N. 1983. Clonal interactions of *Trifolium repens* and *Lolium perenne*. Ph.D. thesis. University of Wales, Cardiff.

TURKINGTON, R. 1975. Relationships between Neighbours among Species of Permanent Grassland (especially *Trifolium repens* L.). Ph.D. thesis. University of Wales, Cardiff.

_____. 1985. Variation and differentiation in populations of *Trifolium repens* in permanent pastures. Pp. 69–82 *in* J. White (editor), Studies on Plant Demography: a Festschrift for John L. Harper. Academic Press, London.

White, J. 1979. The plant as a metapopulation. Ann. Rev. Ecol. Syst. 10: 109–145.
_____. 1984. Plant metamerism. Pp. 15–47 *in* R. Dirzo & J. Sarukhán (editors), Perspectives on Plant Population Ecology. Sinauer Associates, Sunderland, Massachusetts.
Wright, S. 1943. Isolation by distance. Genetics 28: 114–138.
_____. 1946. Isolation by distance under diverse systems of mating. Genetics 31: 39–59.
_____. 1969. Evolution and the genetics of populations, Volume 2: the theory of gene frequencies. Univ. of Chicago Press, Chicago.
_____. 1978. Evolution and the genetics of populations, Volume 4: variability within and among natural populations. Univ. of Chicago Press, Chicago.

BIOLOGICAL IMPLICATIONS OF LEGUMINOUS TREE ARCHITECTURE

Roelof A. A. Oldeman[1]

ABSTRACT

Although the available information on the architecture of leguminous trees barely covers a hundred tree species, it is here arranged and explained according to five interacting organization levels. The leaf-bearing shoots are plagiotropic, orthotropic, or dorsiventral but orthotropic, or constitute short shoots. The branched models, or architectural models, built with these axes are nine, the most remarkable features being the morphological equivalence of all axes, the tendency to form sympodial structures, and the dominance of mixed axes of a plagiotropic/plagiotropic or orthotropic/orthotropic nature. The branched models are rebuilt from meristems in older trees: this gives rise to reiterated, more or less model-conforming crownlets either in an organized way or opportunistically. Considered as a whole organization at several levels, leguminous tree architecture can become so flexible that it can be expressed in a shrub or a liana, even in one species. That its flexibility probably is adaptive is illustrated by a tentative explanation of the growth dynamics of East African *Acacia* trees as linked to the sapstream.

I. INTRODUCTION

Biologists adopt four approaches when they set out to understand the architecture of trees. These approaches may be either static or dynamic and each may be either descriptive or explanatory. In this section, descriptive models and systems hierarchy are treated, and section II will give further explanation and examples.

Tree architecture is an instant picture of a tree, drawn to scale and including only such details as are relevant for the organization of that tree. Details of the organs and of the environment of the tree are omitted. This leads to a descriptive, static, graphic model.

The life of the tree can be represented as its architecture at successive points in time. A sequence of scale drawings may be made of the seedling, the stages of first branching, first flowering, first fruiting, maximal crown expansion, and senescence (Offermans, 1986). Extra drawings can be inserted in the series depicting the organism's development if this is necessary for better understanding. This leads to a descriptive, dynamic, graphic model.

Hallé & Oldeman (1970), Oldeman (1974), and Hallé et al. (1978) established architectural tree models. These models do not represent trees directly in terms of their organs, but have additional levels of abstraction between the level of organs and that of the whole tree. The first of these levels represents the organization of one axis with the organs that it bears. If and when organized at this first level, trees show only one structural aboveground axis, bearing leaves (e.g., seedlings) or leaves and flowers (e.g., certain palms), but also one known unbranched leguminous tree, *Pithecellobium hansemanii* from New Guinea (Hallé et al., 1978).

The second level represents the organization of a branched system—that is, a tree built by more than one of the first-level axes. Trees conforming to such models are constructed by more than one aboveground axis, the branching nearly always resulting from the functioning of axillary meristems. Branching models, in the dynamic approach, grow according to an inherited sequence of either activity or rest of meristems. The term "rest" is used purposely to show that it may include

[1]Department of Silviculture & Forest Ecology, Agricultural University, 6700 AH Wageningen, The Netherlands.

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more than "dormancy." Differentiated meristematic activity, moreover, is programmed to build differentiated axes when a meristem is active. Meristems are thought to ensure two kinds of functions in the growth program. They build axes and direct the behavior of the lateral organs, particularly meristems, on these axes (see Sackville Hamilton, this volume).

At the third level of complexity, the tree system is built by branched subsystems. Meristems can express their potential "outside" the program of the branching sequence, which defines the second level. Adventitious meristems may become active, such as in the "meristem neoformation" in French literature. Functioning meristems may show a rejuvenation of their behavior and start to build an axis closely resembling a seedling axis. Resting meristems may become active and behave in a "younger" way, as can be seen in topped saplings where such meristems form a new trunk. In all cases, this behavior repeats the branching model partly or wholly. Whenever this process begins in a phase young enough to reproduce a trunk and crown, the tree shows "crownlets" inside the total crown. The repetition of the branching pattern by such meristems is so common in trees that it was described formally as "reiteration of the model" (Oldeman, 1974). The reiteration pattern, as a symptom of aging of a large tropical leguminous forest tree species, has been analyzed and demonstrated for *Piptadeniastrum africanum* in Ivory Coast (Offermans, 1986).

Systems hierarchy, as described herein, includes organs as subsystems of organ complex systems, organ complexes as subsystems in branched complex systems, and branched complexes as subsystems in reiterated systems. This is a first step on the road from description to explanation that is presented in section II, below.

The great complexity in the life history of most leguminous trees is evident in that most of them start their growth at the first, unbranched stage as a seedling, then adopt their branched model as a sapling, and finally expand and regenerate their trunks and crowns by the process of reiteration. The life story of *Acacia auriculiformis*, observed by Edelin (1984) in Indonesia, illustrates this process (Fig. 1). This author distinguished an organized, inherited pattern as one manifestation of reiteration, which originally was thought by the author (Oldeman, 1974) to be unpredictable. In trees such as *Acacia auriculiformis*, all main structural meristems assume a "younger" behavior in due time, whereas unpredictable reiteration may be added earlier or later. When sequential branching in the branched model is followed by a predictable sequence of reiteration, Edelin speaks of the trees as showing "architectural metamorphosis."

II. Biological Explanations

Described architecture, growth, and development can be explained in several ways, and this always requires more research time than mere observation. One way of making the step from a descriptive model to an explanatory model is to look for the processes underlying the structures. This implies ecophysiological research in which inputs and outputs between the tree and its environment are measured and analyzed. Such research, as far as we know, does not yet exist, since ecophysiology and production ecology most often concern plant populations. The proportion for each individual tree in the simulated energy and nutrient cycles is not measured but calculated as a weighted average. Such data cannot yet be linked to tree architecture and morphogenesis.

Part five of the book edited by Tomlinson & Zimmermann (1978, pp. 401–532) gives an overview of studies in architectural physiology. The holistic physiological tree model formulated by Schmidt (1980) strongly suggests that sapstream physiology is the most promising way to obtain physiological explanations for tree architecture. A simplified version of this model, as used earlier by Oldeman (1974), is given in Figure 2. Contrary to Borchert's crown–root model (in Tomlinson & Zimmermann, 1978), this model shows three main production systems that have a direct feedback effect on architecture: roots, cambium, and crown. The green mass has an input–output relationship with the environment (energy, gases, water) and with the tree itself (ascending sap = input; descending sap = output). The cambium receives input from the sapstream; its output is transport

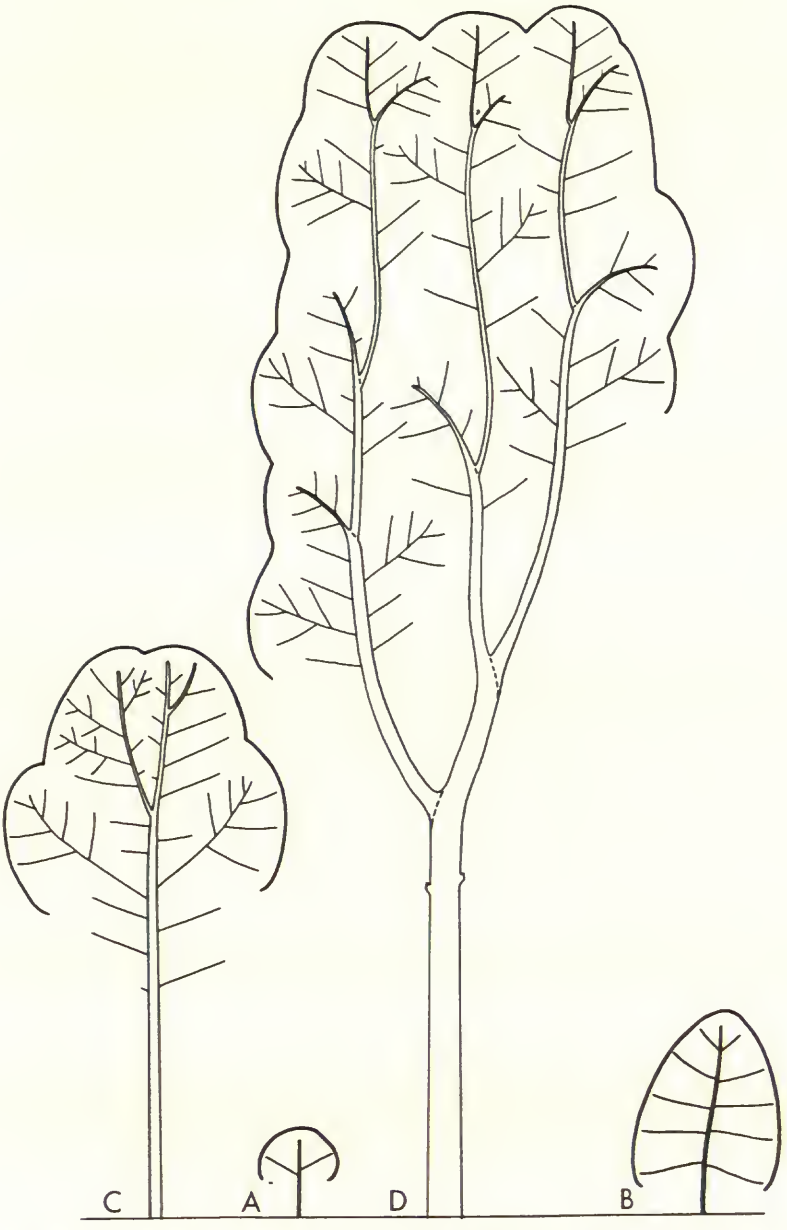

FIGURE 1. The life cycle of *Acacia auriculiformis* after Edelin (1984). —A. Young sapling with orthotropic axes and rhythmic branching. —B. Older sapling with continuous branching. Note monopodial trunk in saplings. —C. Metamorphosis of the architecture by sequential reiteration branches, which bear a model-conform crown (cf. sapling). Note that the branches of the highest order are of the same morphology as those in the saplings and that the "second-order trunk" is interposed between trunk and high-order branches. —D. Fully grown tree, in which model-conform structures behave as if they were mixed axes (cf. Fig. 4H). Flowering not indicated, but occurs on the twigs at the branch extremities. All drawings diagrammatic and derived from scale drawings reproduced in the original publication.

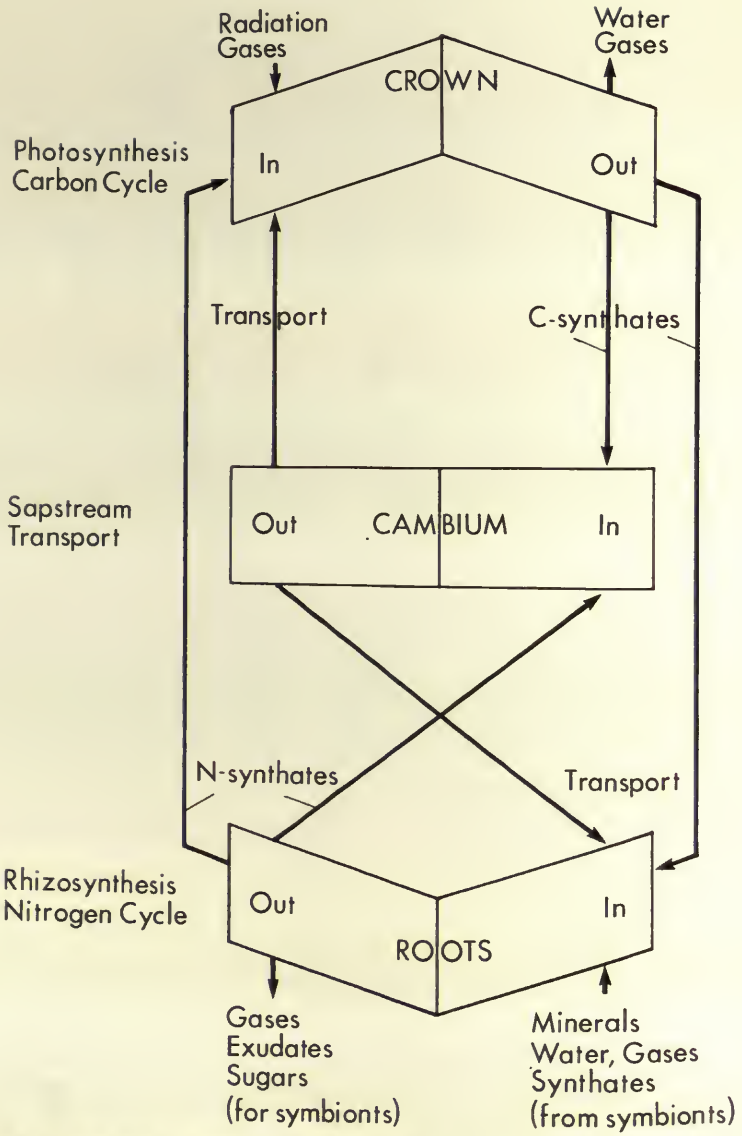

FIGURE 2. Sapstream diagram, simplified and modified after Oldeman (1974). The tree is considered to contain three production compartments, which are in mutual feedback interaction via the sapstream: the crown (photosynthesis, carbon cycle), the root system (rhizosynthesis, nitrogen cycle), and the cambial system (produces transport tissue for the sapstream and derives its nutrients and energy from the sapstream). In principle, this diagram could be simulated by a computer, were enough physiological and ecological data available. This not being the case, the diagram is used to assess the effects of increasing or decreasing inputs from the environment on tree behavior.

capacity for that sapstream, i.e., phloem, xylem, and rays. The root system receives input from the environment (water, minerals, symbiont assimilates, gases), yielding output to that environment (exudates, sugars to symbionts, gases) and to the tree (ascending sapstream). This overall image,

which has been checked in its general lines by using physiological data (Schmidt, 1980), is one element of explanation used below.

To be explanatory, a model has to show a system in terms of its subsystems and their interaction. As shown in the introduction, the life of a tree is so complex that a two-level model is not enough. Hence, the biological significance of the architecture in leguminous trees will be outlined as a hierarchical, five-level hypothesis, in which the precise details of the two extreme levels are omitted. These levels are only considered from the outside, as black boxes. The explanation remains hypothetical, since existing research is insufficient to prove it; therefore care is taken to keep it testable where assumptions are made. These explanations are, in terms of hierarchic systems:

Axial differentiation: combination of axes and their appendages (black box subsystems) in differentiated shoots (systems).

Sequential branching: combination of differentiated shoots (subsystems) in branched, model-conforming trees (systems).

Reiteration of the model: combination of different model-conforming structures (subsystems) in whole trees (systems).

Input- and output-relations between trees (subsystems) and their environment, a black box yielding and receiving matter and energy.

III. Shoot Differentiation

In Leguminosae, all known types of shoot differentiation may be found (cf. Hallé et al., 1978). The four most important ones will be diagnosed herein.

ORTHOTROPIC SHOOTS

Orthotropic shoots (Fig. 3A) are vertically oriented and form and bear their appendages in a spiral that may be conveniently characterized by terms in the Fibonacci series. They are considered as the lowest step in differentiation sequences because most often the youngest organ, the seedling axis, has orthotropic characters.

The plain, orthotropic shoots that may be found in Leguminosae generally bear rather large or very large leaves. Among the largest are the multipinnate ones in Amazonian *Schizolobium* species (Caesalpinioideae). The seedling axis in many *Acacia* species (Mimosoideae) is orthotropic and bears small pinnate leaves, followed by phyllodes, all formed along a spiral. In *Gliricidia sepium* (Papilionoideae), medium-sized pinnate leaves are found on variously bent or curved orthotropic axes.

The extent of orthotropic differentiation can be determined by studying the behavior of cuttings from such shoots: after rooting, they can scarcely be distinguished from seedlings, whether they were taken from the trunk or the branches. No such checks have been made under controlled circumstances of the species cited in this paper.

PLAGIOTROPIC SHOOTS

Plagiotropic shoots (Fig. 3B) are horizontally oriented, form and bear their appendages in two rows represented by the Fibonacci fraction of ½, and are considered as being further differentiated than orthotropic shoots because they are very rare in seedling axes.

Strict plagiotropy, in this sense, is often found in Leguminosae. The large, umbrella-shaped crowns of large forest trees in genera such as *Parkia*, *Piptadeniastrum* (Mimosoideae), or *Voua-*

capoua (Caesalpinioideae) are built by shoots of this type, sometimes with quite large leaves but habitually with medium-sized ones. In Papilionoideae these crowns and axes appear to be rare.

Strict plagiotropy can also be checked by taking cuttings. If these root at all, they yield rooted branches conserving all their plagiotropic characters. In the case of mixed axes (see below), this may be less evident in the subsequent growth pattern of the tree derived from such a cutting.

DORSIVENTRAL, ORTHOTROPIC SHOOTS

These shoots (Fig. 3C) are the result of early or late movement of appendages into a horizontal plane. The shoot apex must be studied in microscopic detail in some cases to check whether the very young leaves or leaf primordia are spirally inserted (as in Papilionoideae), but most often morphological observation will be sufficient to discover petiole torsions or leaf positions indicating that internodes have adjusted their position.

Observations are too few to ascertain the frequency of this kind of dorsiventrality in Leguminosae; it is often found in environments exercising various kinds of stress. It is shown, for instance, on the structural axes in *Robinia pseudoacacia* (Maas & Gubbels, pers. comm.) and many East African *Acacia* species.

The buds on cuttings in such cases form orthotropic shoots which, after rooting, behave exactly like the first seedling axis. Such a rooted cutting can only be distinguished from a seedling if it is still attached to the parent axis. Hence, the differentiation is not physiologically fixed in the meristems as in strict plagiotropic axes.

SHORT SHOOTS

Short shoots (Fig. 3D), as observed in the Leguminosae, are orthotropic with very short internodes, their spiraled leaves forming small umbrellas as in *Acacia* cf. *gerrardii* in Kenya (Fig. 5). The physiology of differentiation in short shoots of the Mediterranean (introduced) *Gleditsia triacanthos* has been thoroughly studied by Neville (1969). He emphasized the "immunity" of short shoots against senescence. In experiments, he succeeded in counteracting the meristematic inhibitions so that short shoots grew into long shoots that showed the normal aging suppressed in the short shoots. Nonleguminous short shoots, as studied by Neville, suggest this to be the general rule in trees that produce these kinds of axes.

EXPLANATION BY FUNCTIONAL AND PROCESS HYPOTHESES

In the years between 1960 and 1980, much experimental work was done on the physiology and functions of the differentiation of leaves, shoots, and whole plants. This work has been summarized and discussed by authors such as Edelin (1977, 1984), Nozeran (1978), or Givnish (1978). The different shoots referred to earlier occur at one end of the sapstream (Schmidt, 1980; Zimmermann, 1983—see simplified version given in Fig. 2). The shoots here act both as sinks and sources.

During their growth and development, the shoots are mainly using the sapstream and consume the many energy-bearing substances that are needed for organ building. Once developed, their role is reversed from a user to a producer. They actively produce and transport photosynthates and hence become positive net sources in the tree.

Following Givnish (1978), one might conclude that building compound leaves is a flexible and economical way to drain the sapstream. With small leaflets, the whole leaf can be finely adjusted to the available amount of energy and matter in the sap. In his study on conifer architecture, Edelin (1977) used a leaf symbol to indicate short shoots. He explained this by referring to the role of such

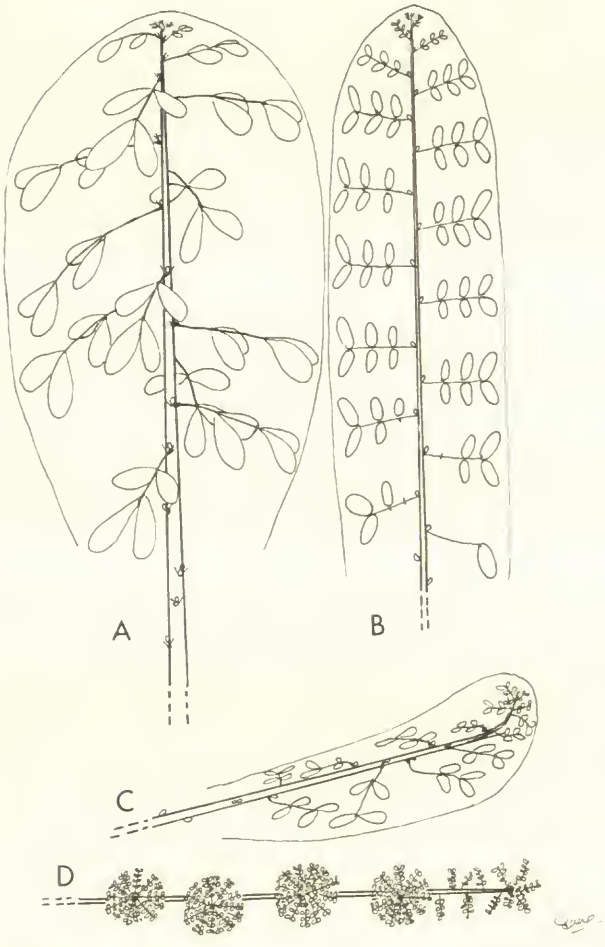

FIGURE 3. Four kinds of leaf-bearing axes as building blocks in leguminous tree architecture. —A. Orthotropic axis, vertical with spirally arranged leaves. —B. Plagiotropic axis, horizontal with distichous leaves. —C. Dorsiventral orthotropic axis, on which dorsiventrality is secondarily obtained by leaf and/or internode torsions. —D. Short shoots such as found in East African *Acacia* species.

shoots as photosynthetic units comparable to compound leaves. This conclusion is accepted here. It may therefore be said that, by virtue of their compound leaves, Leguminosae are flexible at the organ level and that those species which show short shoots (e.g., some *Acacia* spp., *Gleditsia triacanthos*) have a second level of flexibility.

Around Saül, in French Guiana, a common forest tree germinates in open places and grows higher than 40 meters. This is an undescribed species of the genus *Sclerolobium* (Caesalpinioideae; sterile voucher specimens in CAY, *Oldeman s.n.*). Its leaves may become over one meter long, with large leaflets and an apparently indeterminate growth. Most often there is a small cluster of leaflet initials at the top of the rachis. Roux (pers. comm., 1974) established that there is no rachis meristem hidden in this cluster. Hence, this leaf is a normal compound leaf with determinate growth. However, the growth pattern can be interrupted at any moment by blocking the unfolding of the next pair of leaflets, which shows the flexibility in such leaves, the size of which can be

adjusted to the balance between sapstream requirements and environmental inputs. This adjustment is possible by virtue of the leaf architecture. A simple leaf of the same total surface area cannot arrest its development halfway without remaining immature, i.e., it is unable to adjust halfway to a mature production pattern. The permanently young character of short shoots, as established by Neville (1969), shows that at the level of the shoot juvenile characteristics may be combined with optimal adjustment. These functional hypotheses can be tested easily by well-established physiological, morphogenetic, and ecophysiological experimental procedures.

Dorsiventrality in shoots, as described above, is either self-maintained by physiological correlations inside that shoot or imposed by constraints from other shoots (shoot meristems) in the tree (Edelin, 1984, p. 204). According to this author, and based on an extensive bibliography, dorsiventrality in strict plagiotropic shoots or in reversibly plagiotropic shoots is linked to the specialization of such shoots for photosynthesis and flowering. In their environment, they are "exploiting" shoots, whereas orthotropy characterized "exploring" shoots such as the trunk. There is no formal physiological proof of this hypothesis. It tallies, however, with the consequences of the sapstream analysis, particularly with the high investment of matter and energy in vertical woody organs such as the trunk (cf. Odum & Pigeon, 1970). In Leguminosae this image is less simple.

IV. Architectural Models in Leguminosae

The combination of shoots into a branching pattern in leguminous trees is less straightforward than in many other arborescent plant groups. In 1970, Hallé & Oldeman conveyed an image of leguminous trees as a group with two main characteristics, i.e., strict plagiotropic axes and a sympodial branching pattern in which vertical growth is ensured by a succession of meristems. Since 1970, an incredible variety of architectures inside this family have become apparent (cf. Hallé et al., 1978). The information, however, is still too scarce. Therefore, the architectural themes and variations can only be glimpsed as yet, and so the following pages present no more than a rough sketch. Root architecture is omitted altogether here, because the data in this field are so fragmentary that only an anecdotal survey would be possible. A case study is now being done on *Robinia pseudoacacia* roots with the aid of perforons, or root boxes (Bosch, 1984; Maas & Gubbels, pers. comm.).

Architectural Models

Figure 4 represents the architectural models known to occur in at least one species of Leguminosae and is based on the study of about 100 tree species in a family which contains thousands more. Most of the data are summarized in the "architectural files" that are kept at the Institute of Botany (163 rue A. Broussonet, 34000 Montpellier, France). What remains of the Hallé & Oldeman (1970) picture is that more than half of these species grow according to Troll's model (Fig. 4I) and no more than one quarter are built by the growth of a sequence of simple orthotropic or plagiotropic branches. Most leguminous trees show mixed axes in a sympodial sequence.

Mixed Axes

"Very diagrammatically," wrote Hallé & Oldeman (1970, p. 114), "each of the elementary axes shows one basal vertical part, in the rôle of the trunk, and a horizontal terminal part, in the rôle of branch, separated by a curve of variable radius, in the rôle of summit." Many leguminous trees are constructed by the superposition of this kind of building elements, one upon the other. Most often, there is a functional differentiation inside one bending axis, which is either wholly orthotropic or wholly plagiotropic, including the shoots showing a reversible dorsiventrality. Therefore, the two

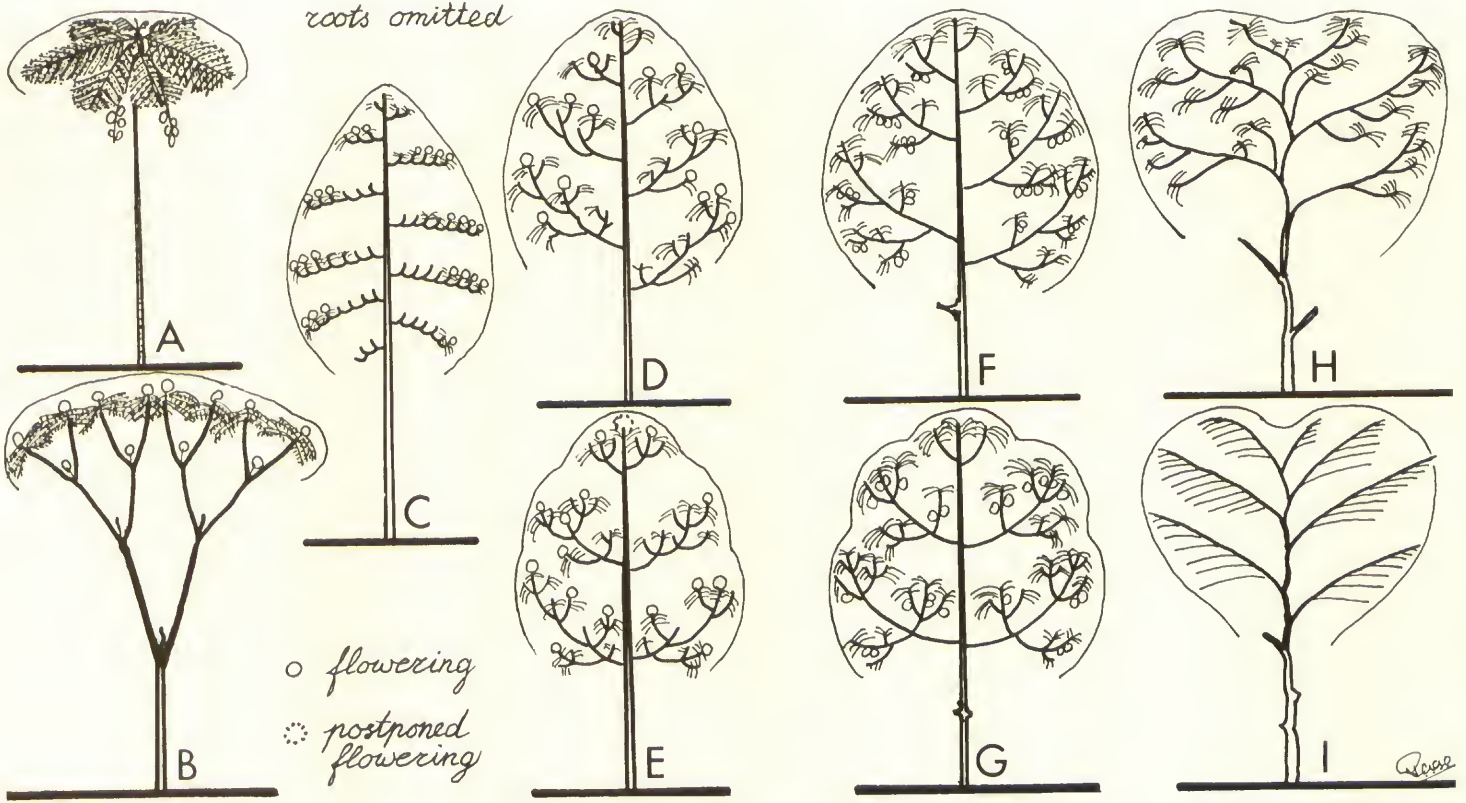

FIGURE 4. Architectural models occurring in Leguminosae (cf. Hallé et al., 1978, for lists of species). —A. Corner's model. —B. Leeuwenberg's model; sympodial, axes orthotropic with terminal inflorescences. —C. Petit's model; all axes orthotropic, branches with orthotropic, terminally flowering reduced modular axes. —D. Stone's model; all axes orthotropic, terminally flowering except (most often) the trunk, branching continuous. —E. Scarrone's model, all axes orthotropic with potential terminal inflorescence (in trunk often postponed) and rhythmic branching. —F. Attim's model; as Stone's model, but inflorescences axillary. —G. Rauh's model; as Scarrone's model, but inflorescences axillary. —H. Champagnat's model; built by mixed axes with an orthotropic base (trunk function) and orthotropic extremity (branch function) separated by a curve on which height growth is sympodially taken over by a subsequent mixed axis of the same type. —I. Troll's model; as Champagnat's model, but mixed axes have a plagiotropic base and a plagiotropic extremity.

first kinds of mixed axes are orthotropic/orthotropic mixed shoots and plagiotropic/plagiotropic mixed shoots.

Champagnat's model (Fig. 4H) is made up of the first kind of shoot, and Troll's model (Fig. 4I) by the second. For the sake of completeness, two other kinds of mixed axes are mentioned here, although they have not yet been found in Leguminosae: orthotropic/plagiotropic mixed shoots (Mangenot's model), and plagiotropic/orthotropic mixed shoots (Oldeman & Hallé, 1980). Because Mangenot's model is often found in shrubs, the architecture of which is all but unknown in Leguminosae, and because the fourth kind of axis shows up in herbs and may well be present in trees showing metamorphosis (Edelin, 1984), these last two kinds of axes are likely to be found in Leguminosae.

SYMPODIAL SUPERPOSITION

Only one example is known in the Leguminosae of a tree growing by superposition of simple orthotropic relay axes with terminal flowering, building Leeuwenberg's model (Fig. 4B). This is *Schizolobium excelsum*, a much-cultivated caesalpinioid tree from Amazonia. Wherever sympodial tree models have been observed elsewhere in this family, they are built with mixed axes. Here, Troll's model (Fig. 4I) is most regular, because plagiotropy is so easily seen as a pattern of leaves, leaf scars, and branches in two rows. Champagnat's model (Fig. 4H) includes wholly orthotropic mixed axes which bend over, the nonvertical parts often showing a semblance of dorsiventrality because the leaves tend toward a horizontal position by petiolar torsions. At first sight the architecture may therefore seem irregular.

In both Troll's and Champagnat's models, the curve in each axis where the vertical base gradually becomes a more horizontal and branchlike extremity is still a puzzling feature. It is no more precisely defined in one model than in the other. Some point on this curve behaves as the apex of the tree, and the lateral meristem closest to that point takes over the growth in height of the tree. The apex of the mixed axis, on the contrary, henceforth plays the role of a mere branch apex. In section V below, this behavior will be discussed when it is displayed not by axes, but by whole model-conforming structures.

EXPLANATION BY SYSTEMS HIERARCHY, COMPARISON, AND PROCESS HYPOTHESES

Systems (here the branching model) and subsystems (here the shoots) always interact. The model requires the right kind of axes to be built, but on the other hand it causes these axes to develop certain characteristics. Straightforward orthotropic and plagiotropic shoots are known to occur as individual plants. This is not so for mixed axes, at least not in trees. These are only known as parts of branched architectures, even if they are in some cases unique and central parts (e.g., certain *Annona* [Annonaceae] trees; also in herbs such as *Medicago lupulina*, Papilionoideae [Jeannoda-Robinson, 1977, p. 49]). In small trees and herbs, the existence of one single mixed axis, moreover, is attributed to the evolutionary loss of such axes in "miniaturization" of an original model (Hallé et al., 1978).

The comparison of all architectural models in leguminous trees does not in itself elucidate the causes of the clear tendency to build Troll's and Champagnat's models. There are too few examples, and theory in this field remains highly hypothetical because not enough experimental work has been done. Both a sufficiently complete architectural data base for at least one large genus and experiments that are designed with these data in mind are needed. The thorough architectural study of 30 Guianan species of *Inga* (Mimosoideae) by Poncy (1985) reported that all those species show Troll's

model. *Cassia* s.l.[2] (Caesalpinioideae), a genus with species conforming to several models, will be used to illustrate some explanatory hypotheses.

Referring to Figure 4, cassias are known to grow according to the following models:

Scarrone's model (Fig. 4E): *Cassia* [*Senna*] *alata, C.* [*Senna*] *siamea.*

Rauh's model (Fig. 4G): *Cassia* [*Senna*] *occidentalis* (a woody herb, 75 cm high).

Champagnat's model (Fig. 4H): *Cassia* [*Senna*] *chrysocarpa, C.* [*Senna*] *quinquangulata,* and other climbing cassias from the Guianas.

Troll's model (Fig. 4I): *Cassia grandis, C. javanica, C.* [*Chamaecrista*] *mimosoides,* and *C.* [*Senna*] *multijuga.*

In all *Cassia* s.l. seedlings seen by the present author, there are some traces of orthotropy, even if it was only for the first few internodes where the cotyledons and first leaves are not distichous. Hence, it may be assumed that all cassias have in common an initial orthotropic differentiation phase, even if it is ephemeral.

Cassia [*Senna*] *alata* is noteworthy for its relatively massive axes, large compound leaves, and its spectacular yellow subterminal inflorescences, axillary just below the apical meristem that aborts. The whole structure functions as if it were a terminal inflorescence (pers. observ. in Ivory Coast, 1986). The treelet does not always branch, but if it does, the lateral axes are orthotropic, massive, and bear the same kind of subterminal inflorescences. They are formed in tiers; branching is rhythmic. When decapitated, the stump yields sucker shoots (i.e., it reiterates) with the same characteristics. This may be compared with the architecture of a much-cultivated tree, *Cassia* [*Senna*] *siamea.* Both species follow Scarrone's model, but in the second species there is a tendency to postpone flowering, so the axes have a prolonged vegetative growth period and flowering occurs terminally on higher-order branches. This makes for a potentially larger tree, and indeed *Cassia* [*Senna*] *siamea* is a pioneer tree growing much larger than *C.* [*Senna*] *alata,* a pantropical ruderal shrub.

In *Cassia* [*Senna*] *occidentalis,* flowering is shifted in space but not in time. This plant produces lateral inflorescences on the orthotropic axes, which show a rhythmic branching pattern, and hence conforms to Rauh's model, but it remains a small undershrub that flowers early. Its axes are remarkably slender, and the reduced cambial activity is to be noted.

Cassia [*Senna*] *chrysocarpa* and other climbers of the genus (with their showy yellow terminal inflorescences) are built by long orthotropic axes, and also have reduced cambial activity. These axes flower terminally, also on laterals that are morphologically equivalent to the main axis and issue from meristems located on bends of the first axis; this is Champagnat's model (Fig. 4H). The axes are much longer and thinner in climbers than the illustration shows.

Cassia grandis, a large forest tree from the Guianas, was observed for over six years in the Cayenne ORSTOM Botanical Garden. The seedling has a short and ephemeral orthotropic stage and then becomes strictly plagiotropic. With its distichous leaves, the axis may reach a height of one to two meters. Somewhere in the curve from vertical to horizontal (lower down in shaded plants and higher up in plants exposed to full light), growth in height is taken over by a lateral axis that is plagiotropic from the beginning. This last pattern has been established for other forest tree species (*Cassia javanica* and *C.* [*Chamaecrista*] *mimosoides*). *Cassia* [*Senna*] *multijuga,* observed only as a hedge plant, has this same architecture. The whole behavior of the axes indicates a differential cambial activity, postponed at the top but increasing at the base, and the overall pattern is that of Troll's model.

The key word in *Cassia* s.l. seems to be postponement of either secondary thickening or flowering or both. Postponement is a purely relative notion, referring to the "least postponing" species

[2]The author here treats *Cassia* in the broad sense, not following Irwin & Barneby (*Mem. New York Bot. Gard.* 35: 1–918. 1982), who accept three genera (*Cassia, Chamaecrista,* and *Senna*) and revise them for the Americas. The placement of a species in either *Senna* or *Chamaecrista* by Irwin & Barneby is noted in the text.

known in the group (i.e., *Cassia* [*Senna*] *alata*). It has to be noted also that no rhythmic meristematic activity has been observed in this assemblage. Rhythmic growth in an axis is the alternation, accompanied by differentiation, of meristematic and cambial activity or leaf development. This is a form of rather rapidly alternating postponement, which in the longer term occurs in the branched model as a whole.

The postponement of secondary thickening is an important process in woody Leguminosae. Genera such as *Bauhinia* (Caesalpinioideae), *Pterocarpus* (Papilionoideae), or *Mimosa* (Mimosoideae) each contain trees or treelets showing Troll's model. Lianas in *Bauhinia* are clearly plagiotropic, with their bandlike climbing stems placed in a plane perpendicular to that of the distichous leaves. Climbing species of *Mimosa* are equally clearly built by orthotropic axes. No detailed analysis is available, however, for these climbers of *Mimosa* or *Pterocarpus*.

The experimental work on plagiotropy by Roux (1968) indicates that plagiotropy includes tendencies to determinate growth patterns, thinner axes, and a shorter lifespan. In other words, with increasing plagiotropy, leaf-bearing shoots converge toward compound leaves, at least physiologically. This can be checked by comparing the behavior of leaf cuttings with that of plagiotropic branch cuttings.

The longer flowering and secondary thickening are postponed, the better are the conditions for a lianescent life cycle; that is, the more flowering is relegated to the youngest parts of the shoots and thickening to the oldest parts. In fact, transitions between treelike and lianalike forms occur in "weeping" trees. For instance, Akoa (1980), when considering weeping forms in trees, gave photographic documentation of a weeping *Cassia javanica* introduced in the Cameroons. In this context, he wrote on certain "pseudolianescent" phenomena in weeping trees. In Champagnat's model, trees and shrubs probably do just this, with a balance between extension growth and secondary thickening that maintains just enough rigid sustaining tissues to keep the axes upright, such as in *Gliricidia sepium*, a shrub from the liana-rich Papilionoideae. In other cases, which seem to be of more rare occurrence, large trees can be built in this way (e.g., the neotropical *Andira* of the same subfamily). Some East African *Acacia* trees (Mimosoideae) start in this way, but continue their life cycle with more complications (Fig. 5).

Troll's model, because of its plagiotropic axes, relegates most of its photosynthesis toward the plagiotropic branches at the extremity of a mixed axis, whereas in Champagnat's model the photosynthetic surface consists of clusters or cylinders of spirally arranged leaves. Although both models may turn lianescent, the flattened and highly organized leaf layers in Troll's model can intercept light over larger surfaces with less investment in energy or nutrients. A genus like *Inga* (Mimosoideae) includes species from early pioneers (e.g., *Inga edulis*) to high forest trees (e.g., *Inga bourgonii*). All *Inga* species observed are built strictly according to Troll's model (Poncy, 1985), but the genus does not contain climbers as far as is known to the present author. The availability of light at each stage of the life cycle should be an important factor in explaining the functioning of Troll's model in comparison with that of Champagnat and can be checked easily under experimental conditions.

The main fact that emerges from the consideration of branched architectural models in Leguminosae is the structural flexibility that predominates in the trees of this family. This flexibility arises from a number of properties of their growth patterns:

All models (Fig. 4) are built by morphologically equivalent axes, with the exception of Corner's model (Fig. 4A) and one observed shrub in the genus *Sesbania*. The fact that every meristem will yield the same kind of axis increases the probability of successful regeneration after damage.

The predominance of mixed axes among leguminous tree species introduces a balance between extension growth and secondary thickening that can easily adjust to the available environmental resources according to the feedbacks of the sapstream diagram

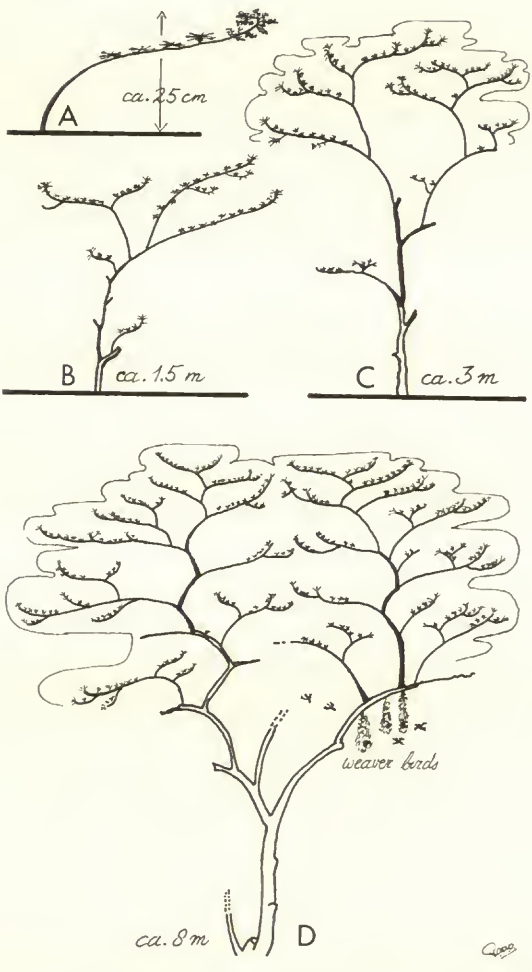

FIGURE 5. Schematic representation of the architecture during the life cycle of East African *Acacia* species, from Oldeman (in press). Development stages in alphabetical order, described in text. Stars = short shoots. Kenya, April 1981.

(Fig. 2). The level and shape of the curved portion of a mixed axis can therefore be considered at least partly to be determined exogenously.

Terminal flowering blocks the possibility of monopodial vegetative growth on the same axis and leads to sympodial development of a plant. This also occurs in an architecture with mixed axes, where the position of the inflorescences does not matter because mixed axes form sympodia in any case. Together, models with sympodial growth account for more than 90% of the leguminous trees observed up to the present. This increases the flexibility of growth dynamics when added to the morphological equivalence of all axes.

The combination of these properties makes for a flexibility that allows several species to escape the limits of one biological type. *Pterocarpus officinalis*, often a large forest tree, has been observed at the borders of mangroves in the Ile de Cayenne (French

Guiana) as a healthy flowering shrub. *Spirotropis longifolia* (Sophoreae) produces both lianas and trees, and in the Kew greenhouses the tree *Xanthocercis zambesiaca* (Sophoreae) was transformed into a liana by pruning the main axis at the age of two years (Stirton, pers. comm.). Ornamental *Bauhinia* lianescent species can be grown as healthy shrubs and hedges when cut back. The available observations suggest (but do not prove as yet) that many leguminous species should be described not as *being* trees or climbers but as *behaving* as such biological types in well-defined vegetation types.

The existence of experimental research on leguminous branching patterns and architectural models is not known to the present author. Such research will require physiological rigor, bearing in mind the warning by Zimmermann (pers. comm., 1975) that the closed circuit of the sapstream causes experimental inputs to have effects everywhere in the tree sooner or later. It will also demand carefully measured or controlled environments, because many leguminous trees adjust their architecture so finely to the environmental resources that in certain cases they may not be trees at all but take other life forms.

V. Reiteration and Metamorphosis

Reiteration, according to Hallé et al. (1978, p. 269), is defined as: "Any modification of the tree's architecture not inherent in the definition of its model and which is occasioned by damage, environmental stress or supraoptimal conditions. Reiteration usually involves the bringing into activity of resting apical meristems not normally involved in the expression of the model, but can also involve change in the orientation of a shoot, e.g., from plagiotropy to orthotropy."

Edelin (1984) discovered modifications of the tree's architecture that were not normally inherent in the definition of its model, were not due to direct effects of environmental impacts, and were predictable. He wrote (Edelin, 1984, p. 233): "The existence of 'automatic' reiteration leads us to think that a plant cannot indefinitely increase its size while maintaining the same way of functioning. Once it arrives at some 'limiting volume,' here defined by a maximum order of branching, it doubles its vegetative apparatus and conserves, grafted upon itself, the new 'organisms' to which it has given birth. In this sense, perhaps it obeys the same biological laws as those which determine the division of cells or the budding of a hydra?" Edelin's meaning is clearly referable to Figure 1, which graphically summarizes his large file of observations on growth, development, and architecture in *Acacia auriculiformis,* an Australian tree studied in Indonesia that shows more than one aspect of architectural flexibility. To begin with, it shows rhythmic branching as a young sapling, but then it rapidly changes over to branching in a continuous spiral (Fig. 1A, B), all axes being equivalent by orthotropy. Because it does not flower at this age, it cannot as yet be classified in one of four branching models distinguished by their flowering pattern (Fig. 4D–G). None of these models show mixed axes; the young tree has a monopodial, orthotropic trunk. This is why the species is included in Edelin's study on monopodial trees.

Having reached a certain developmental stage that, following Edelin, can vary considerably according to the age and size of the tree, the original model is reiterated at the place of certain branches, the apical meristems of which begin to function accordingly (dedifferentiation: a branch meristem functions as a trunk meristem; Fig. 1C). When the tree grows still higher, the extremity of the original trunk bends over, height growth is taken over by one of the branched models somewhere on the curve, and this process continues (Fig. 1D).

Mixed Models

A comparison between Figures 1D and 4H shows a striking similarity in general pattern. In Figure 4H, orthotropic axes bend over, and a young axis of the same type is formed in the curve

as a relay in a series of such axes. In Figure 1D, it is a whole branched model that behaves in this way: its base remains part of a sympodial trunk, and its branched crown henceforth functions as a complex branch. In fact, the same kind of organization as on the lower level has been achieved but at a higher level of complexity.

It is interesting to ponder the importance of such shifts in architectural complexity from one organizational level to another, from a subsystem to a system. Such shifts have been mentioned earlier in photosynthesis, from leaf to short shoot, in flowering by postponement or displacement, and in secondary thickening. The whole image conveys an extraordinary and general flexibility of leguminous growth dynamics and architecture, if it were confirmed to be representative for the whole family.

The "building blocks" of trees with a degree of complexity comparable with *Acacia auriculiformis* in its fully grown state (Fig. 1D) are "mixed models," not mixed axes. If these models were themselves built by mixed axes, it would become extremely difficult to distinguish where the original branched model ends and where reiteration begins. Such a gradual blending between manners of organization that, in many trees, represent two distinct levels of complexity would add one more flexibility ingredient to the overall architecture and growth dynamics.

The acme of this kind of architecture is the wholly plagiotropic mixed axis with the potential for wholly plagiotropic mixed laterals. By virtue of this building block—which can vary considerably in age, size, and proportions—no organization barriers exist any more between the axis, the branched model, and the reiterated tree. It is both highly organized at the level of the plagiotropic mixed axis and highly opportunistic, because wherever the tree's survival requires additional biomass, it can be added in the form of one mixed axis or a sympodial series of such axes.

The development series in *Piptadeniastrum africanum* given by Offermans (1986) is a documented example of just this phenomenon. Built by plagiotropic mixed axes, and conforming to the classical definition of Troll's model (Fig. 4I) when young, the tree continues to build both large and small crowns in all places, at all levels, and at all moments when necessary. These crownlets cannot be distinguished on morphological criteria as being either part of the original model or models added by reiteration. However, by a process of clustering on those axes that show a high rate of secondary thickening, postponed of course, the mixed axes form partial crowns inside the total tree crown. These partial crowns behave like individual tree crowns and even show "crown shyness" between them, a parameter for aging. Crown shyness is the phenomenon of crowns, in this case reiterated crowns, that keep a distance from one another, so that one perceives light between them when looking upwards. Explanations of crown shyness, as advanced in the literature, can be found in Offermans (1986). These patterns have been analyzed by Offermans in digital terms on detailed aerial photographs of tree crowns.

SOME TERMS OF EXPLANATION

Too little information is available on complete architectural life cycles of leguminous trees. Therefore, no explanation offered here can be representative for all woody Leguminosae. There is enough evidence, however, to suggest some meaningful testable hypotheses and general observations at the level of the fully grown and aging tree.

> All axes are equivalent, and in metamorphosed trees all models are equivalent. This can be tested by observation on many more species but not explained without parallel experiments. The simplest of these concern cuttings. Less simple experiments would entail measurements of the sapstream at different levels and chemical analysis of the assimilation process per mixed axis and per partial crown.

> The architectural flexibility of Leguminosae should be tested in conditioned environments and in the field by precise measuring of environmental gradients, including soil

gradients. Such experiments take a very long time if one tree is to be monitored, hence they should be broken up in time by using coexisting individuals in different development phases, and in space by bearing on precise metabolic processes of certain axes.

With the functional separation between the tree apex, a curve in an axis, and the axial apex, simple explanations based on apical dominance do not apply to many leguminous trees. The concepts of apical dominance and the derived ones have been developed when studying trees with one monopodial trunk. Another physiological model will have to be developed, maybe in the direction sketched by Champagnat (1978).

VI. Adaptation to Environment

It may be assumed that the complex and flexible architecture and growth dynamics of leguminous trees allow them to survive in complex and flexible environments. Architecture should not be thought to be the only set of characters that is not adaptive. A simple case study will serve to illustrate that there are many indications to support the hypothesis that indeed architecture contributes to the adaptation of the tree to its environment.

The life cycle of some East African *Acacia* species based on preliminary field observations in Kenya on *Acacia drepanolobium, A. gerrardii, A. nilotica, A. melliflora,* and an unknown variety of *A. senegal* has been represented in Figure 5. The species were named in the field by MAB-UNESCO experts; there was no possibility of collecting reference specimens for the herbarium. *Acacia drepanolobium* has been cultivated in the greenhouse in Wageningen over several years. Due to the lack of light and perhaps the short day length in winter, and the inability to heat over 18°C, this species could be observed under stress. Under these conditions, it dropped its short shoots, confirming that these organ complexes behave with some similarity to leaves (see above discussion on short shoots in Section III). In the following spring, new leaves and short shoots were formed.

All of the *Acacia* species listed in the previous paragraph are built by orthotropic axes. The seedlings were not like those of *A. auriculiformis* (Fig. 1) but consisted from the beginning of a mixed axis. Short shoots appear at an early stage on the seedling axis, which is the first one in a sympodium conforming to Champagnat's model. This sympodium often shows more than one relay axis on the curve of an older mixed axis. Crownlets are formed on the older wood. They have the same model, but the basic segments of the mixed axes in this position remain very short. In this way the crownlets together determine the well-known umbrellalike physiognomy of the East African *Acacia* trees. Their axes never acquire strict plagiotropy. This can be observed at the apex, where the young leaves are clearly spiraled. In the more horizontal parts, secondary dorsiventrality occurs on these axes, particularly visible by the dorsal position of the short shoots, which appear a decimeter or more behind the apex. Flowering takes place on twigs of a high order, mainly short shoots, as can be seen on the photographs of *A. xanthophloea* and *A. drepanolobium* published by Hargreaves & Hargreaves (1972).

This example has been given to show that the growth dynamics of leguminous trees in stressed circumstances (e.g., the severe dry season in East Africa) tally with an explanation based on the sapstream diagram (Fig. 2). Although the root system should hold an important place in such explanations, the data are lacking to include it. Therefore, whenever underground inputs are mentioned, these are based on an educated guess.

If inputs from the environment were unlimited, East African *Acacia* architecture would have no adaptive sense. Rain in these regions often occurs in showers separated by dry periods. The input of water comes in through the roots and then stimulates the formation of more green mass and wood (Fig. 2, feedback arrows). This stimulus being short-lived, it has to be processed rapidly. There are certainly other biological solutions to achieve this, but the *Acacia* species do it by: rapid formation of leaves, with a minimal investment at the shoot level in wood (short shoots); minimizing the

structural woody parts needed for mass sap transport by reducing the size of basal parts of mixed axes at the level of the branched model; spreading the crown so the interception of light for the processing of ascending sap is maximized and by the formation of many crownlets on the branch segments of the mixed axes, at the reiteration level which can barely be separated from the model; and adjusting the leaf surface by building small pinnate leaves with minute leaflets at the organ level. The same strategy is probably also effective when biotic stress is applied (e.g., by fungi, parasites, or herbivores).

The huge *Acacia* root systems mentioned in the literature can be explained in this model as buffers for the unreliable water supply. Their symbiosis with microorganisms contributes to this function, and the whole argument is strengthened when the soil lacks fertility, as it often does in African savanna regions.

The capacity to coppice easily, which is common to many leguminous trees with this or a comparable life cycle and architecture, is one more response to the severe stresses that can occur (e.g., fire). Moreover, it points toward another buffer that has been omitted in Figure 2. This is the stock of reserve nutrients in wood and roots that is present in all trees and that, among other things, feeds coppice shoots. All processes mentioned can be checked and experimentally studied. Other species, described before, indeed can be explained by the sapstream diagram if one varies the inputs.

VII. Conclusions

As a first conclusion it has to be stated that the architectural study of the Leguminosae is not yet ripe to yield general rules that can be substantiated. Treating the biological implications of leguminous tree architecture hence remains a provisional undertaking.

A survey of the available information still yields an image that can be tied to other data from studies on legume species and from biological theory in general. Even given only as a sketch which still lacks precision, the following picture emerges.

1. The Leguminosae contain trees with a very complex architecture as well as a few trees that seem to be built in a more simple way.
2. Tree architecture in legumes tends to be very flexible, which agrees with their geographical distribution and occurrence in numerous very diverse environments.
3. The biological type "tree" may be only one expression of the potential architecture in a species, which also may grow as a shrub or a climber.
4. The very flexibility of tree architecture in the Leguminosae makes it quite difficult to study by physiological and ecophysiological experiments.
5. Simple physiological theories, such as apical dominance, are mostly too narrowly defined to cover the growth dynamics of the Leguminosae.
6. The flexibility of tree architecture in the Leguminosae seems to be an adaptive property. This is tentatively explained by using a sapstream diagram.

References

Akoa, A. 1980. Processus d'obtention de certaines structures adultes chez les arbres tropicaux. Ph.D. thesis. University of Yaoundé, Cameroon.

Borchert, R. 1978. Feedback control and age-related changes of shoot growth in seasonal and nonseasonal climates. Pp. 497–515 *in* P.B. Tomlinson & M. H. Zimmermann (editors), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Bosch, A. L. 1984. A new root observation method: the perforated soil system. Oecolo. Pl. 5:61–74.

Champagnat, P. 1978. Formation of the trunk in woody plants. Pp. 401–402 *in* P. B. Tomlinson & M. H. Zimmermann (editors), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Edelin, C. 1977. Images de l'architecture des Conifères. Ph.D. thesis. University of Montpellier, France.

———. 1984. L'architecture monopodiale: l'exemple de quelques arbres d'Asie tropicale. D.Sc. thesis. University of Montpellier, France.

Givnish, T. J. 1978. On the adaptive significance of compound leaves, with particular attention to tropical trees. Pp. 351–380 *in* P. B. Tomlinson & M. H. Zimmermann (editors), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Hallé, F. & R. A. A. Oldeman. 1970. Essai sur l'architecture et la dynamique de croissance des arbres tropicaux. Masson & Cie, Paris. [English translation by B. C. Stone, Penerbit Univ. Malaya, Kuala Lumpur, 1975.]

———, R. A. A. Oldeman & P. B. Tomlinson. 1978. Tropical Trees and Forests: an architectural analysis. Springer-Verlag, Berlin, Heidelberg, New York.

Hargreaves, D. & B. Hargreaves. 1972. African Trees. Hargreaves Company, Kailua, Hawaii.

Jeannoda-Robinson, V. 1977. Contribution à l'étude de l'architecture des herbes. Ph.D. thesis. University of Montpellier, France.

Neville, P. 1969. Morphogénèse chez *Gleditsia triacanthos* L. III. Etude histologique et expérimentale de la sénescence des bourgeons. Ann. Sci. Nat. Bot. Biol. Végétale 10, 3: 302–324.

Nozeran, R. 1978. Multiple growth correlations in phanerogams. Pp. 423–443. *in* P. B. Tomlinson & M. H. Zimmermann (editors), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Odum, H. T. & R. F. Pigeon (editors). 1970. A Tropical Rain Forest. 3 volumes. Office Inform. Serv. U.S. Atomic Energy Commiss. Oak Ridge, Tennessee, U.S.A.

Offermans, D. H. J. 1986. Crown shyness: a parameter for aging in *Piptadeniastrum africanum*. Netherlands J. Agric. Sci. 34: 493–497.

Oldeman, R. A. A. 1974. L'architecture de la forêt guyanaise. Mém. Office Rech. Sci. Techn. Outre Mer 73. Paris.

———. Elements of Silvology. Springer-Verlag, Heidelberg, Berlin, New York (in press).

——— & F. Hallé. 1980. Sobre los ejes mixtos plagio-ortótropos en algunos árboles tropicales. Misc. Pap. Landbouwhogeschool Wageningen 19: 281–287.

Poncy, O. 1985. Le genre *Inga* (Légumineuses, Mimosoideae) en Guyane Française: systématique, morphologie des formes juvéniles, écologie. Mém. Mus. Natl. Hist. Nat, Sér. B, Bot. 31.

Roux, J. 1968. Sur le comportement des axes aériens chez quelques plantes à rameaux vététatifs dimorphes. Le concept de rameau plagiotrope. Ann. Sci. Nat. Bot. Paris, Sér 2, 9: 109–256.

Schmidt, P. 1980. Bomen over groei van bomen. Chapter 4 *in* R. A. A. Oldeman, Grondslagen van de Bosteelt. LH-Bosteelt, Wageningen, The Netherlands (out of print).

Tomlinson, P. B. & M. H. Zimmermann (editors). 1978. Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Zimmermann, M. H. 1983. Xylem Structure and the Ascent of Sap. Springer-Verlag, Berlin, Heidelberg, New York.

STRUCTURE AND EVOLUTIONARY TENDENCIES OF INFLORESCENCES IN THE LEGUMINOSAE

F. Weberling[1]

ABSTRACT

The basic ramification type of the inflorescences in Leguminosae is a heterothetic double (or multiple) raceme ("pleiobotryum" of Troll). This implies that their morphology follows the polytelic type in the sense of Troll. Presently, only two exceptions, *Gleditsia triacanthos* and *Gymnocladus dioicus*, are known in which the main axis ends in a terminal flower. If this flower is not pseudoterminal, both inflorescences must be regarded as monotelic. In *G. triacanthos*, triadic or dyadic branches can occur at the very base of the racemoid inflorescence. These features could point to a derivation of the polytelic Leguminosae inflorescences from a (monotelic) paniclelike inflorescence as represented frequently within the related families of Rosidae. Dyadic or triadic branches at the base of the racemelike inflorescences are also reported for *Sophora japonica* and *Cladrastis kentuckea* and demonstrate that the prophylls of the flowers, which are typically present in the inflorescences of Leguminosae, can be fertile. Heterothetic double racemes can be observed in all of the three subfamilies. The racemose form of the coflorescences can be modified into a spike, an umbel, or a globose head. In some taxa the number of flowers of the florescences is reduced to one single flower. The branching system of many ligneous Leguminosae is determined by the differentiation of long shoots (macroblasts), growing with long internodes and serving as a main axis, and lateral short shoots (brachyblasts), in which the internodes remain (often extremely) short, often with the exception of the flower-bearing axes. In these systems, the production of inflorescences is generally restricted to the brachyblasts. As in other families, flowering of brachyblasts can be retarded for several seasons, resulting in different forms of cauliflory. Brachyblasts with truncate synflorescences can also continue their growth by proliferation, finally forming a terminal bud, which can give rise to a new fertile and proliferating growth unit in the next season.

The basic type of branching in inflorescences of the Leguminosae is a heterothetic double (or multiple) raceme (pleiobotryum) in the sense of W. Troll (1964–1969: I, 45). This implies that their morphology follows the polytelic type in the sense of Troll (1964–1969: I, 149). Thus the raceme terminating the branching system of the inflorescence of *Sophora japonica* in Figure 1A represents the *main florescence* (mf), whereas the many-flowered branches are *paracladia* (pc) ending in racemose *coflorescences* (cof), which may be preceded by a zone bearing paracladia of second order (pc′) with coflorescences of a second order (cof′), etc. In this way the paracladia repeat the structure of the main axis of the flowering system, as is suggested by their name ("repeating branches"). The whole flowering system, therefore, is composed of florescences, which together form a *synflorescence*. The whole paracladia-bearing part of the flowering system—the *enrichment zone*—is usually preceded by a zone in which the axillary buds of the main axis remain more or less undeveloped (see also Fig. 1B). In perennial plants this *inhibition zone* is preceded by an *innovation zone*. The axillary buds of the latter zone usually are dormant until the next vegetation period. They give rise to *innovation shoots* of the same architecture as the previous flowering shoots. In woody plants, however, the inhibition zone may be missing. In such cases the innovation shoots arise immediately below the paracladial zone of the previous flowering shoots. The whole and often more vegetative part below the main florescence comprising the paracladial zone, the inhibition zone, and the innovation zone is called "Unterbau" (*hypotagma* = "foundation"). The same consequently applies to the paracladia, which of course have no innovation zone.

[1]Universität Ulm, Abt. für Spezielle Botanik (Biologie V), Postfach 4066, Oberer Eselsberg, D-7900 Ulm, West Germany.

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MONOGR. SYST. BOT. MISSOURI BOT. GARD. 29:35–58. 1989.

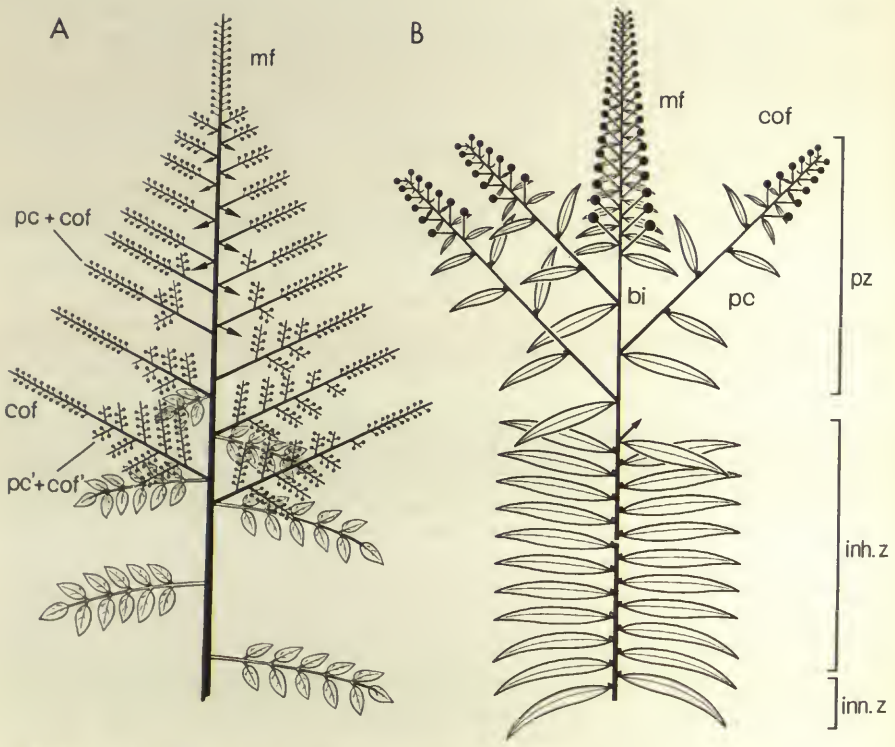

FIGURE 1.—A. *Sophora japonica*, diagram of a synflorescence.—B. Generalized diagram of a polytelic synflorescence; mf = main florescence, bi = basal internode, pc, pc′ = paracladia of first and second order with coflorescences (cof, cof′) pz = enrichment zone (paracladial zone), inh. z = inhibition zone, inn. z = innovation zone, as accessory buds or branches. Original Troll & Weberling (unpublished).

The racemose form of the coflorescence can be modified by an abbreviation of the pedicels and thus be transformed into a spike. Their main axis can also remain short, the florescence becoming umbellate as, for example, in *Inga myriantha* (Mimosoideae, see Taubert, 1891: 101, fig. 55A) or *Lotus*, or—if the pedicels also remain short—resulting in a headlike contraction of the florescence, as, for example, in *Trifolium* or many *Acacia* species.

In the Leguminosae as a whole, there are so far only two cases known, both in Caesalpinioideae, *Gleditsia triacanthos* (Fig. 2A, B) and *Gymnocladus dioica* (*G. canadensis*, Fig. 24A), in which the main axis ends in a terminal flower (see also P. Neville, 1958: 53, fig. 9). Therefore, both inflorescences must be regarded as *monotelic*. The inflorescences of *Gleditsia triacanthos* are also of interest because of the occurrence of triadic (or dyadic) branches at the very base of the inflorescence (Fig. 2C; Troll, 1961). These features of the inflorescence of *Gleditsia triacanthos* could point to a derivation of the polytelic legume inflorescences from a (monotelic) paniclelike inflorescence as represented frequently within the families of the Rosoideae.

Dyadic or triadic flowering branches at the base of the racemelike inflorescences are not limited to *Gleditsia triacanthos*. They are also reported (Troll, pers. comm.) for *Sophora japonica* (Fig. 3B–D) and *Cladrastis kentukea* (Fig. 4D, E), and demonstrate that the prophylls of the flowers, which are typically present in the inflorescences of Leguminosae, can be fertile.

The first one or (mostly) two leaves of a branch are designated by a special term, *prophyll(s)*,

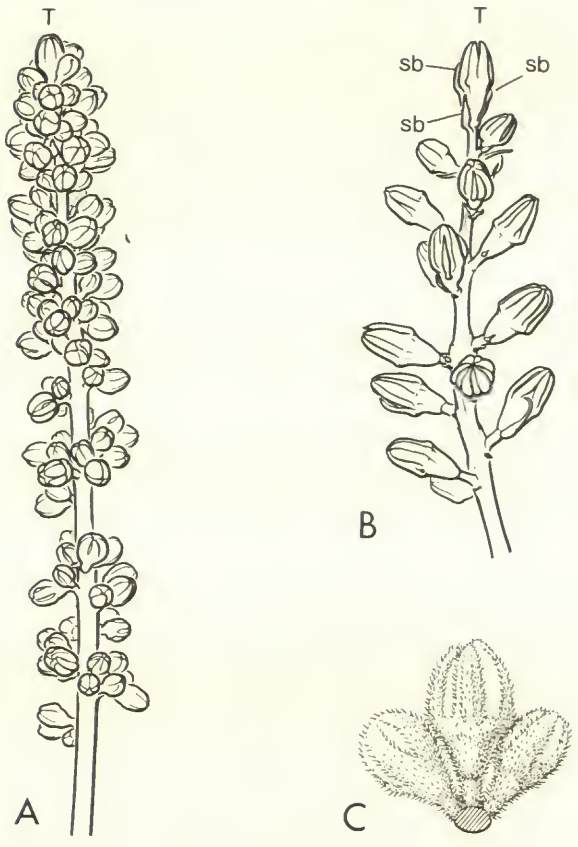

FIGURE 2. *Gleditsia triacanthos*.—A. Male inflorescence.—B. Female inflorescence.—C. Triadic partial inflorescence from the base of a male inflorescence. sb = sterile bracts ("Zwischenblätter," fide Troll) below the terminal flower (T). Original Troll (unpublished).

because of their characteristic position and feature, which often differs from that of the following leaves. In case of two prophylls (α and β-prophyll) these often occur in a transverse position. The internode preceding the prophylls (or the first of them, the α-prophyll), which is the first internode of the branch, is called the *hypopodium;* the one following the prophylls is the *epipodium*. If both prophylls are separated by an internode, it is called a *mesopodium*.

Heterothetic double racemes can be observed in all three subfamilies: sometimes in *Adenanthera* (Mimosoideae); in *Cynometra, Copaifera officinalis* (Fig. 5), *Hymenaea courbaril* (Caesalpinioideae); and in the Sophoreae and many other taxa of the Papilionoideae (*Amorpha*, Fig. 6A). In these cases the main florescence is the first to flower; the sequence of flowering of the paracladia can be basipetal or divergent (Sell, 1980).

In *Sophora japonica* (Fig. 1A) and *Dalbergia thomsonii* (Fig. 7A) the series of paracladia show a continuously increasing degree of ramification toward the base of the synflorescence, whereas the distal paracladia are confined to their (co)florescence and have no hypotagma. In *Lupinus*, however, the main florescence is predominant and the development of paracladia is often suppressed (Fig. 8A). If they ever unfold and flower, even the distal paracladia are characterized by an extensive sterile hypotagma below the coflorescence (Figs. 6B, 8B; see also Troll, 1964–1969: I, 224). There

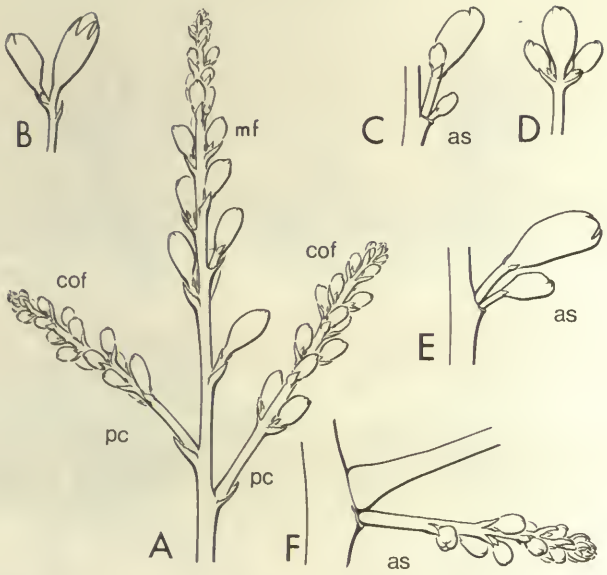

FIGURE 3. *Sophora japonica*—A. Distal part of a synflorescence.—B. Dyadic. C, D. Triadic partial florescence in side view (C) and from the abaxial side (D). The triadic branch in C and the single lateral flower in E are accompanied by accessory flowers (as):—E. Base of a paracladium (pc) accompanied by an accessory branch which bears a terminal florescence. mf = main florescence, cof = coflorescence. Original Troll (unpublished).

are some examples in which the flowering system is restricted to the terminal raceme, for example in species of *Caesalpinia, Cytisus nigricans* (Fig. 9C), *C. sessilifolius,* and in *Genista sagittalis* inter alia.

In many taxa of the Leguminosae, however, the inflorescence lacks a terminal main florescence, and this gives rise to a so-called "truncate" polytelic synflorescence (Troll, 1961; 1973: 103–104). It occurs in most Mimosoideae (*Acacia* inter alia, many Caesalpinioideae, e.g., the species of *Cassia* [Fig. 10A]) and very many Papilionoideae, as in the well-known genera *Trifolium* (Fig. 11; see Wydler, 1860, 1872), *Vicia, Lathyrus, Melilotus* (Figs. 12, 13), *Medicago,* and many other herbaceous and woody taxa. In *Medicago lupulina* rare cases are known in which a main florescence was formed. According to Sell (1980), "in *Lespedeza bicolor* . . . most often there is a terminal raceme, but sometimes on particularly long shoots that carry very many lateral racemes the top exhausts itself without producing a terminal raceme."

In truncate polytelic synflorescences of the Leguminosae a specialization of the paracladia can be observed (Figs. 12, 13): the distal paracladia (pc) are restricted to their (co)florescences (cof) and bear no hypotagma, and taken altogether they form an indeterminate homothetic ("homothetic" because all the racemose florescences are lateral; Troll, 1964–1969: I, 45) double raceme comprising the distal part of the whole synflorescence. The subjacent paracladia repeat this structure. They also do not end in a (co)florescence but develop a series of paracladia of second order (pc′), consisting of coflorescences of second order (cof′) only. These two forms of paracladia are distinguished by Troll (1965: 99) as short and long paracladia.

Continuation of vegetative growth is also maintained in rosette plants, where the corresponding apex remains indeterminate, producing frondose leaves which, perhaps following a certain periodicity, bear long paracladia or short paracladia in their axils. Both types of paracladia can remain

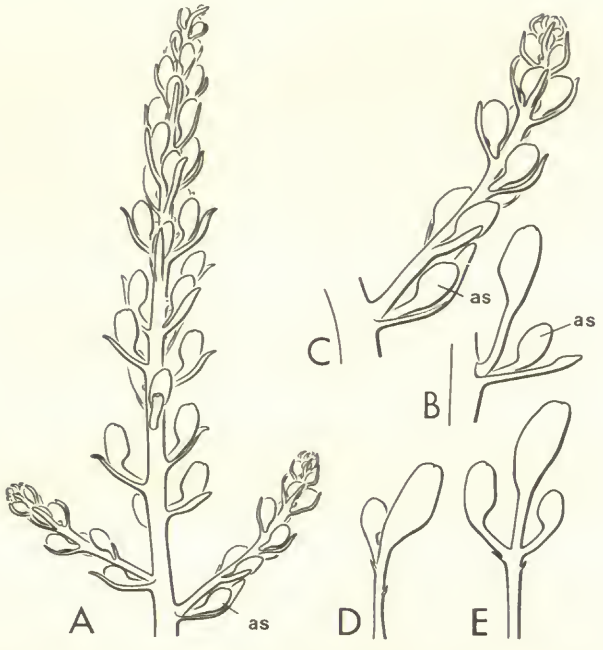

FIGURE 4. *Cladrastis kentukea* (syn. *C. lutea*).—A. Distal part of a synflorescence.—B. Single lateral flowers, accompanied by an accessory flower.—C. Paracladium with coflorescence, accompanied by an accessory flower (as).—D, E. Dyadic and triadic partial florescences. Original Troll (unpublished).

short, with the exception of the hypopodia of the florescences, which develop as peduncles. It is possible to imagine that this growth form is derived from growth forms like those of *Melilotus* (Figs. 12, 13) if the main axis of the whole system remains short. Examples of the resulting rosette growth form can be found in many species of the genera *Astragalus* and *Oxytropis* (for example, *Oxytropis campestris* [Fig. 14A] or *O. halleri*). Other species in these genera, like *Oxytropis pilosa, Astragalus alpinus,* and *A. frigidus,* have an elongate main axis.

In *Astragalus exscapus* even the peduncles (the hypopodia of the coflorescences) remain short; thus a sessile umbel-like aggregation of flowers is formed (Fig. 14B). Growth forms with shortened main axes can vary in many ways. In *Trifolium repens,* for example, long paracladia originating in the axils of the frondose leaves of the main rosette form "runners," which grow with long internodes, form roots at their nodes, and bear long-peduncled, upright, globose florescences (see also Wydler, 1860, 1872). Especially in those taxa in which the subtending bracts of the racemose paracladia are leafy (frondose), the indefinite structure of the synflorescence allows the plants "to reconcile strong vegetative growth in length with abundant flowering" (Sell, 1980: 287).

Another possible way in which indeterminate synflorescences can develop is through a complete return to vegetative growth. This takes place regularly, and often repeatedly, in the flowering branches of some woody taxa like *Robinia, Caragana* (Fig. 24B), and species of *Colutea* (Fig. 15; Troll, 1961: 89). This phenomenon, called *proliferation,* also is the reason why the flowering zone in some climbers such as *Vicia cracca* or *Lathyrus pratensis* can be interrupted by one or more vegetative zones (Troll, 1973: 104).

Proliferation also can affect the apex of racemose florescences. Thus in *Lupinus polyphyllus* it may occur that at the end of the flowering period the apex of the main florescence passes over to form frondose leaves instead of bracts. This is also reported for the main florescence of *Cytisus*

FIGURE 5. *Copaifera officinalis*, showing heterothetic double racemose partial inflorescences. After Köhler (1923, t. 171).

nigricans (Irmisch, 1851: 674; Wydler, 1860: 28). This retarded proliferation, which occurs late in the development of an inflorescence (Fig. 9A, B), may be distinguished from cases in which proliferation takes place before the main florescence is formed. Consequently, it may be called premature proliferation ("Früh-" and "Spätprolifikation"; Troll, 1960: 116; 1964).

FIGURE 6.—A. *Amorpha fruticosa*, flowering shoot.—B. *Lupinus luteus*, flowering shoot. Original Troll (unpublished).

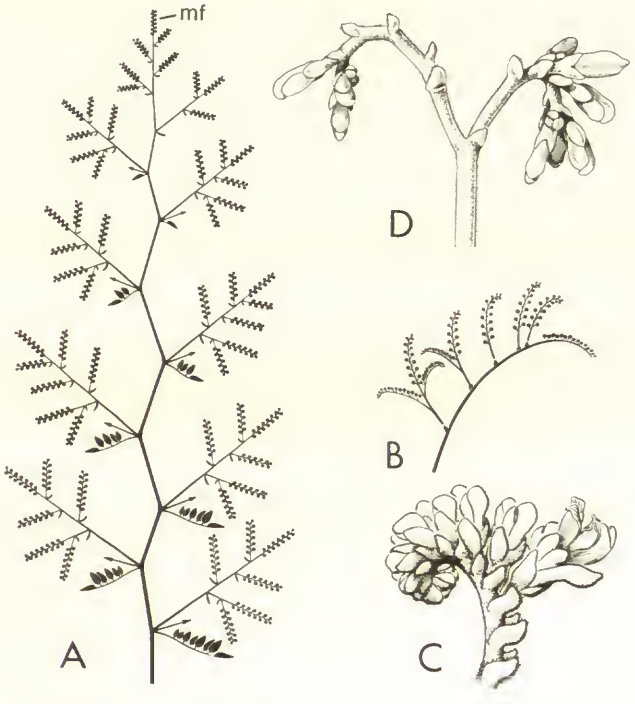

FIGURE 7. *Dalbergia thomsonii*.—A. Diagram of a synflorescence.—B. Distal part of the synflorescence in side view, showing the unilateral arrangement of the flowers.—C. Paracladium.—D. Distal part of a synflorescence. Original Troll (unpublished).

FIGURE 8. *Lupinus polyphyllus.*—A. Flowering shoot.—B. Flowering long paracladia. Original Troll (unpublished).

As a general rule, in the angiosperms the shape of the inflorescences can be modified fundamentally by the bracteose or frondose ("leafy") character of the foliation. This also applies to the Leguminosae, in which the leaves subtending the single flowers within racemose florescences are generally bracteose. One of the exceptions is *Baptisia perfoliata* with proliferating florescences (Fig. 16) in which the yellow flowers contrast very well against the large green bracts.

A peculiarity of the florescences of many Leguminosae, especially among the Papilionoideae, is dorsiventrality, or their "unilateral formation" respectively. "Unilateral formation" (Goebel, 1882) is caused late in development by twisting of the flower stalks, which are originally situated all around the florescence axis, as in *Colutea, Hedysarum,* and *Dalbergia* (Fig. 7B–D). "Dorsiventrality" in a strict sense is the result of an early dorsiventral development because the flower primordia originate prevalently or exclusively on one side of the axis of the florescence. Dorsiventrality occurs, for example, in many Vicieae. *Vicia cracca* has been studied by Goebel (1882, 1928). Here, as in *Trifolium lupinaster* (Trecul, 1854; Goebel, 1882, 1928), the flowers originate only on the abaxial side (in relation to the main axis of the dibotrytic inflorescence) of the florescence primordium; the same applies to *Lathyrus pisiformis* in the basal part of its florescences (Taubert, 1891: 82). In young stages the florescences of *Vicia cracca* are curled toward the axial side, as are those of several *Dalbergia* species (*D. melanoxylon, D. brasiliensis* [see Taubert, 1891: 82, fig. 45C], and *D. thomsonii* [Fig. 7B–D]), the latter showing a certain similarity of curling like the "boragoids" of *Heliotropium* and other Boraginaceae (see Troll, 1965: 103). Especially in *Vicia cracca* the dorsiventrality of the florescences corresponds with the dorsiventrality of the whole flowering shoot. Many additional examples have been investigated by Schneider (1925), who also found many transitional forms as, for example, some *Trifolium* species in which the flowers (at least in the basal part of the florescence) are partly developed on the abaxial side only. Toward the distal end of the florescence, the florescence axis may be completely surrounded by flowers (see also Wydler, 1860; Dutailly, 1877).

FIGURE 9. A, B. *Teline canariensis* (syn. *Cytisus canariensis*), flowering branch; the florescence terminating the main shoot has resumed vegetative growth, producing frondose leaves and branches before returning to the production of flowers.—C. *Cytisus nigricans*, flowering shoot; the synflorescence is restricted to the main florescence.

It is doubtful whether in all cases the dorsiventral or unilateral shape of the florescences confers advantages for pollination. The same applies to the so-called "strobilaceous inflorescences" ("Zapfeninfloreszenzen," see Goebel, 1931) of *Flemingia strobilifera, Desmodium pulchellum* (and other species of this genus), and in *Geissaspis (G. cristata)*, which are characterized by imbricated scalelike bracts or even completely resupinated bracts *(Flemingia, Desmodium pulchellum)*. Since Troll (in Goebel, 1931) found many insects that used the densely packed bracts for shelter while eating flower buds and fruits, Goebel (1931: 234) stated that *Flemingia* might have been better off if it had retained the normal pattern of the Papilionaceae. Certainly the dense flower heads of *Liparia* and other taxa are functional units, but except in some cases like the ornithophilous "bottle-brush flowers" of *Calliandra* or the likewise bird-pollinated *Clianthus* (pollinated by Meliphagidae), very little is known about the role of inflorescence structures in pollination (see Schrire, this volume).

Well known is the contrast of colors in the drooping spikes of the African mimosoid *Dichrostachys cinerea*, which originate from the axils of foliage leaves of brachyblasts ("short shoots"). The proximal flowers of the spikes are sterile but provided with showy mauvish pink sterile stamens, whereas the distal flowers are fertile, with yellow stamens. The similar and likewise dichogamous inflorescences of *Parkia* are bat-pollinated (Hopkins, 1884: figs. 3, 6). Those of *P. auriculata* bear a girdle of sterile nectar-producing flowers between the basal showy flowers and the distal zone of fertile flowers. The basal flowers emit a musty smell appropriate to the attraction of bats (Vogel, 1968).

The inflorescences of *Amherstia nobilis* with their bright, large, red-colored prophylls and red flowers are spectacular, but it is not yet known whether they attract pollinating birds or butterflies.

FIGURE 10. Truncate polytelic synflorescences of *Cassia lanceolata* (A) and *Ononis breviflora* (B). In the latter the uniflorous florescences (*) of the paracladia are exceeded by accessory branches forming long paracladia with lateral pc', each comprising a uniflorous (co)florescence. Original Troll (unpublished).

The subtending leaves of the paracladia can be bracteose, especially in short paracladia, but can also be frondose. Thus, the whole synflorescence may be frondose or frondo-bracteose.

Phylloscopic *accessory branches* (accessory shoots) are very common in the Leguminosae. They often occur as single accessory flowers accompanying a single lateral flower (Figs. 3E, 4B), a dyadic or triadic branch (Fig. 3C), or a racemose paracladium (Fig. 4A). They can also form racemes (Fig. 3F) or vigorous branches with a higher degree of branching than the major branch, as in *Melilotus* (Fig. 13). In species like *Ononis breviflora* (Fig. 10B) the accessory branches that accompany the short paracladia develop into vigorous, long paracladia. They far exceed the short paracladia, which form reduced florescences only. In *Genista germanica* the production of flowers is normally restricted to the phylloscopic accessory branches of thorny, axillary brachyblasts. These accessory branches develop one year after the appearance of the brachyblasts and bear racemose florescences at their ends (see Irmisch, 1848).

Groups of serial accessory buds—not collateral buds as Taubert (1891: 83) erroneously stated—occur in several taxa. They are especially frequent and numerous in Mimosoideae (for example, *Mimosa, Calliandra, Albizia,* or *Acacia* [Fig. 17A, B, D]), where they can form zigzag groups of alternating, stalked spicate, ovoid, or globose florescences of racemose structure.

In some taxa of Papilionoideae the number of flowers in the racemose or spicate coflorescences are reduced to a single flower. This is the case, for example, in *Cicer arietinum, Lathyrus sativus, L. nissolia,* and other species of this genus, *Stylosanthes* (Taubert, 1890), *Cytisus scoparius, Pisum* (Parkin, 1914), and *Tetragonolobus maritimus (Lotus siliquosus).* Species of the genus *Vicia* show transitions between racemes with many flowers *(V. cracca)* and those with one flower *(V. sativa).* In *Ononis* this character has become prevalent within the sections *Acanthononis, Bugrana,* and *Natrix* (see Willkomm & Lange, 1880). Parkin (1914: 554–556) pointed to the fact that in *O. natrix*

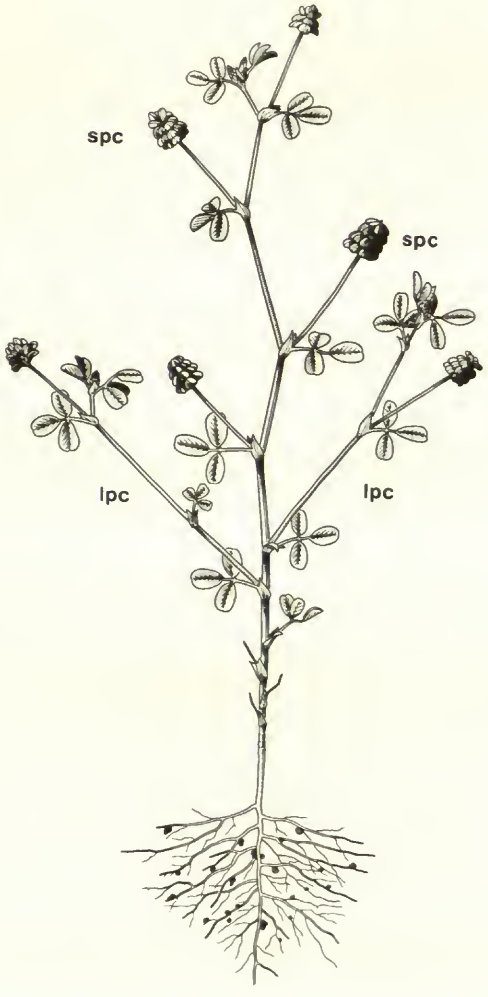

FIGURE 11. *Trifolium campestre*. Flowering plant with short paracladia (spc) and long paracladia (lpc). After Troll.

the manner in which the single flower is attached "shows unmistakable evidence of having been derived by reduction" from a multiple-flowered raceme, as in *O. rotundifolia,* because the flower "is not borne directly on the main axis (peduncle) issuing from the leaf axil, but on a secondary axis (pedicel) arising laterally from the former." The peduncle continues as a short sterile process beyond the point where the pedicel is attached (see also Fig. 18D). The same can be observed in *O. breviflora* (Fig. 18C), or in two-flowered racemes of *O. rotundifolia* (Fig. 18A). In *O. natrix* sometimes a bract demonstrates the lateral position of the single flower (Fig. 18B). For *O. arvensis,* Parkin (1914) reported that the single flower "appears to be borne directly on a short stalk proceeding from the leaf axil" (Fig. 18F). The same can be seen in *O. alopecuroides* (Fig. 18K). In *O. arvensis,* however, two-flowered racemes occur as well (Fig. 18E). The whole series of reduction stages is explained by the schematic presentation in Fig. 18G–K, starting with an expla-

FIGURE 12. *Melilotus officinalis*.—A. The distal zone of the homothetic double raceme of short paracladia of the synflorescence shown in B.—B. Synflorescence with distal zone of short paracladia (spc) and proximal zone of long paracladia (lpc). After Troll.

nation of the architecture of a flowering shoot and the position of the short and long paracladia of *O. rotundifolia* in Fig. 18G (see also Wydler, 1872: 234).

Apart from the conditions mentioned above, reductions and compression of florescences occur in many taxa of the family and in various ways. Some of them may be explained by a series of examples shown in Figure 19 A–C, which should not be interpreted as a morphological series of related taxa. In all of these examples the flowering shoot ends in a truncate synflorescence in which the paracladia consist of the (co)florescence only and have no hypotagma. The florescences are few-flowered in *Sutherlandia frutescens* (Fig. 19A) and originate in the axils of frondose leaves on a main axis with well-developed internodes. In *Psoralea pinnata* (Fig. 19B) the paracladia are densely arranged. Their main axes remain short, thus giving the many-flowered florescences the shape of sessile umbels. The inflorescence of *Tephrosia grandiflora* (Fig. 20) is sharply contrasted against the vegetative zone of the flowering shoot by the bracteose foliation. The flowers appear to be paired ("pseudocymae") because the main axes of the paracladia remain short as in *Psoralea pinnata* and the florescences are reduced to two flowers (Fig. 19C), including a small bud between them which represents the abortive distal part of the florescence. The subtending bracts of the two flowers are shifted above the middle of the pedicels by recaulescence.

A higher degree of complexity of synflorescences with the same ramification mode of the flower-bearing parts is represented by *Lespedeza thunbergii* (Fig. 19D). Here there is no terminal homothetic double raceme composed of paracladia bearing two-flowered sessile racemes, as in *Tephrosia grandiflora* (Fig. 19C). But *L. thunbergii* has a widely extended zone of numerous paracladia without hypotagma, which follows the same mode of ramification as the apical synflorescence of *Tephrosia grandiflora* (Figs. 19C, 20).

The branching system of many woody Leguminosae is determined by the differentiation of long

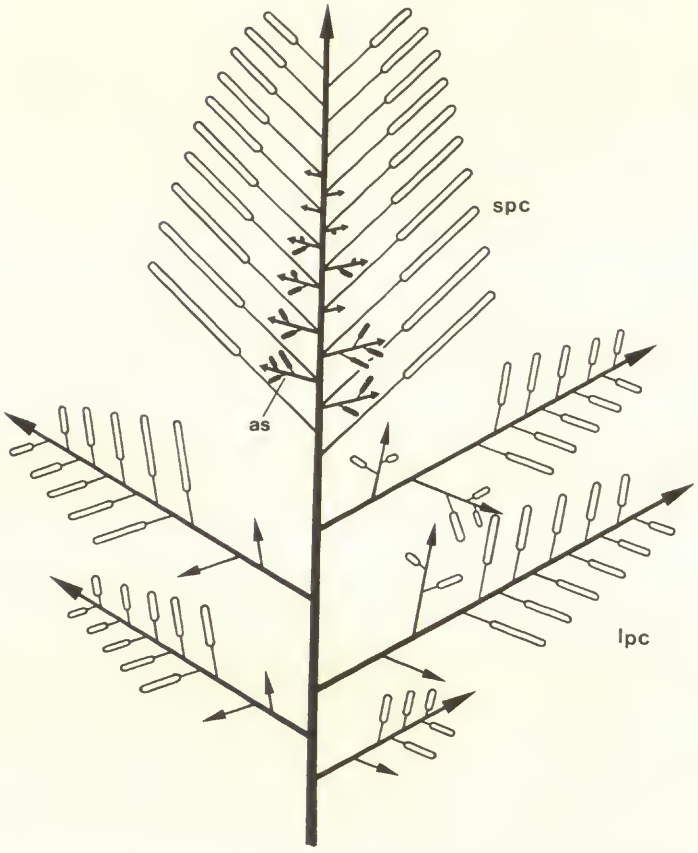

FIGURE 13. *Melilotus officinalis*. Diagram of synflorescence with accessory shoots (as). spc = short paracladia; lpc = long paracladia. After Sell (1980) modified.

shoots (macroblasts), growing with long internodes and serving as a main axis, and lateral short shoots (brachyblasts) in which the internodes remain (often extremely) short, often with the exception of the flower-bearing axes. In these systems, the production of inflorescences is generally restricted to the brachyblasts, which originate from the leaf axils of a previous year's long shoot or of older long shoots. This usually occurs in *Laburnum anagyroides* (*Cytisus laburnum*; Fig. 21B), although even the seasonal long shoot can develop a terminal florescence toward the autumn (Irmisch, 1851). The foliation of the brachyblasts starts with one or two frondose leaves (Fig. 22), followed by a series of bud scales. Above the bud scales, frondose leaves can appear in the next season, which again are followed by bud scales; this can be repeated during several years. Usually the brachyblasts of *Laburnum anagyroides* remain vegetative for two years before they produce a terminal florescence. Thus the synflorescence of the brachyblast is exclusively represented by the main florescence. This usually is preceded by frondose leaves (Figs. 21B, 22). It is not uncommon, however, for a flowering brachyblast to bear scalelike leaves only (Figs. 21A, 23; Troll, 1970: 89). Buds of brachyblasts can also be produced at the ends of the macroblasts, thereby finishing their growth as long shoots. These terminal brachyblasts usually flower in the season after their inception. After flowering, the development of the brachyblast can continue by the unfolding of second

FIGURE 14. Flowering plants of *Oxytropis campestris* (syn: *Astragalus campestris*) (A) and *Astragalus exscapus* (B). The inflorescences originate in the axils of frondose leaves (+) of the indeterminate main shoot. Original Troll (unpublished).

order brachyblasts which arise from buds that originate in the axils of the uppermost leaves below the terminal inflorescence.

As in other families, there are also woody taxa of Leguminosae in which the flowering of brachyblasts can be retarded for several seasons, resulting in different forms of cauliflory. *Cercis siliquastrum* (Caesalpinioideae) is a good example for demonstrating the relation between this phenomenon and the "normal" mode of flowering. The first occasion in which flowering can start is the anthesis of the (main) florescences terminating the brachyblasts of the previous year's long shoot. Since the foliation of these brachyblasts consists of bracteose leaves only, they resemble the bracteate brachyblasts occurring in *Laburnum anagyroides* (Fig. 23). The unfolding of these brachyblasts, however, can be suppressed for many years. Thus flowers appear on old branches and even on the stem. Since the brachyblasts can ramify, probably by development of innovation shoots from their very bases, cushions of flowers can appear on old branches.

On the other hand, brachyblasts with truncate synflorescences can continue their growth by proliferation, finally forming a terminal bud that can give rise to a new fertile and proliferating

FIGURE 15. *Colutea arborescens*. Proliferating synflorescence. After Taubert (1891), corrected by Troll (unpublished).

growth unit in the next season. An example is the brachyblasts of *Caragana arborescens* (Fig. 24B). These have passed several flowering periods, yet still bear the stalks of the axillary florescences of the last season in the axils of withered leaves below their terminal bud. The frondose inflorescence often looks like a simple proliferating raceme. It is composed, however, of highly reduced, often one-flowered, racemose coflorescences. The seasonal shoots arising from the terminal buds can also develop into long shoots, a phenomenon which is also known from other long shoot–short shoot systems.

A specialization of the ramification system into vegetative and flower-bearing shoots can also occur in herbaceous plants, as is shown by the Australian "upside-down peabush," *Brachysema (Leptosema) daviesioides* and *B. chambersii*, in which flowering branches originate separately from the subterranean parts of the plant.

The typology of inflorescences, as elaborated by Troll & Weber (1955) and Troll (1964–1969), enabled Troll (1974) to clarify the diverse structures of flowering systems within the large genus *Acacia*. All species of *Acacia* have truncate polytelic inflorescence systems in which the florescences form racemes, spikes, or globular heads. The most complete form of these truncate polytelic synflorescences is represented by the section *Botryocephalae* (Fig. 25B), in which the florescences are globular. In comparison to the complete polytelic synflorescences in Figure 25A, which ends in a main florescence forming a terminal raceme, the synflorescence in Figure 25B has lost this terminal raceme. It consists of the paracladial zone only, which is divided into a proximal zone of long paracladia and a distal zone of short paracladia that lack a hypotagma. Thus the distal part of

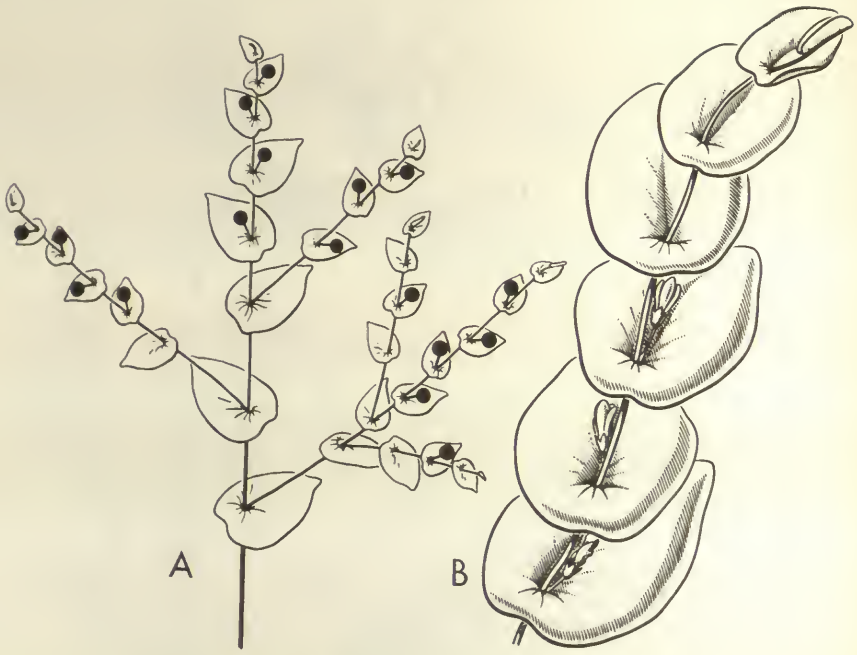

FIGURE 16. *Baptisia perfoliata.*—A. Diagram of a synflorescence.—B. Proliferating florescence. A, after notes by Troll; B, after Curtis's Bot. Mag. 58, 1831, pl. 3121.

the main shoot forms a homothetic double raceme instead of a heterothetic one as in Figure 25A. *Acacia dealbata* is cited by Troll as a good example of this architecture.

As demonstrated in Figure 25C, D, truncation can go even further—a process called "super-truncation" or truncation of second (Fig. 25C) or third (Fig. 25D) degree. For the latter, an example is *A. retinodes* (sect. *Phyllodineae*.) The second degree of truncation may be demonstrated here by *A. cyanophylla* (Fig. 25E, F), where the synflorescence normally consists of long paracladia that form homothetic double racemes (Fig. 25F), the florescences being globose. Sometimes, however, a terminal double raceme representing the zone of short paracladia still occurs (Fig. 25E). Another group of species belonging to the Phyllodineae is characterized by a long shoot versus short shoot differentiation. In this group, called Dicephalae by Troll, the production of flowers is limited to the brachyblasts, which usually bear only two paracladia (Fig. 25G) forming globose florescences, as in *A. dodonaeifolia*, or spicate florescences, as in *A. longifolia*. The brachyblasts of the latter are very similar to the flowering systems of *Albizia lophantha* as shown in Figure 17E. Sometimes the resting bud, which is hidden between the florescences at the top of the brachyblast, can produce more florescences. On the other hand, in *A. dodonaeifolia* the apex of the brachyblast later can expand to produce a leafy macroblast.

The shape of the flowering shoots can be modified to a considerable extent by the occurrence of accessory flower-bearing branches. As already mentioned, especially in *Acacia*, they often occur as a zigzag sequence of many alternating branches (Fig. 17A, B, D) bearing a terminal spicate, ovoid, or globose main florescence. In the production of the main florescence they differ from the truncate character of the whole synflorescence, in which the main florescence is completely lost. At the base of vigorous accessory branches with frondose leaves there can even originate many-headed synflorescences that also terminate in a main florescence.

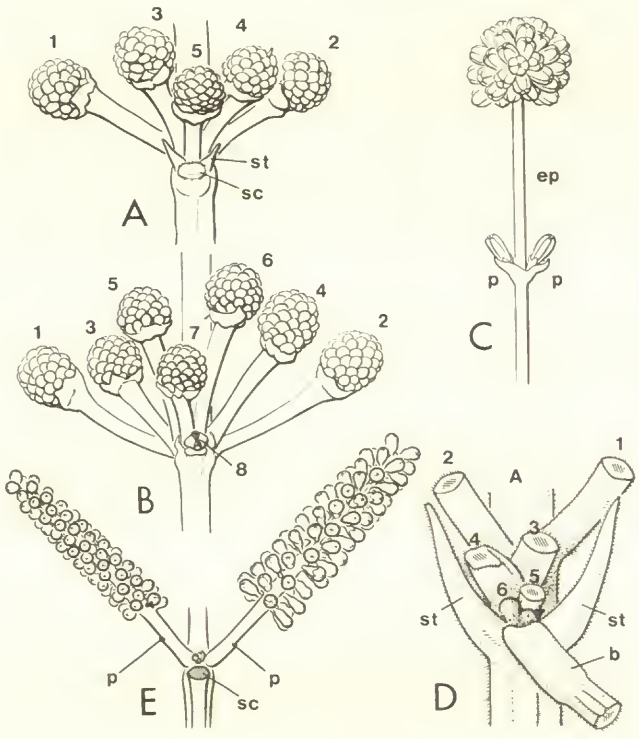

FIGURE 17.—A, B. *Acacia karroo*. Paracladium (l) with accessory flowering branches (2–5 or 2–8). sc = scar of subtending leaf, st = the small thorny stipules still present. C, D. *Acacia arabica*.—C. Paracladium with well-developed epipodium (ep), p = prophylls.—D. Dissected peduncles (hypopodia) of the paracladium (l) and phylloscopic accessory flowering branches (2–5) and two further accessory buds (6, 7) in the axil of the subtending leaf (b). A main axis.—E. *Albizia lophantha* brachyblast with two florescences originating from the axils of the prophylls (p) of the brachyblast, which are shifted nearly to the middle of the peduncles by recaulescence.

To attain a complete general view of inflorescence structures in the large family of Leguminosae and its different tribes and genera, it will be necessary to check in which mode the fundamental structures and their variations, as well as the diverse evolutionary tendencies in the alterations of these structures, are manifested in each taxon. In this way it will be possible to evaluate results of inflorescence morphology for systematics, as well as for physiological aspects and floral biology.

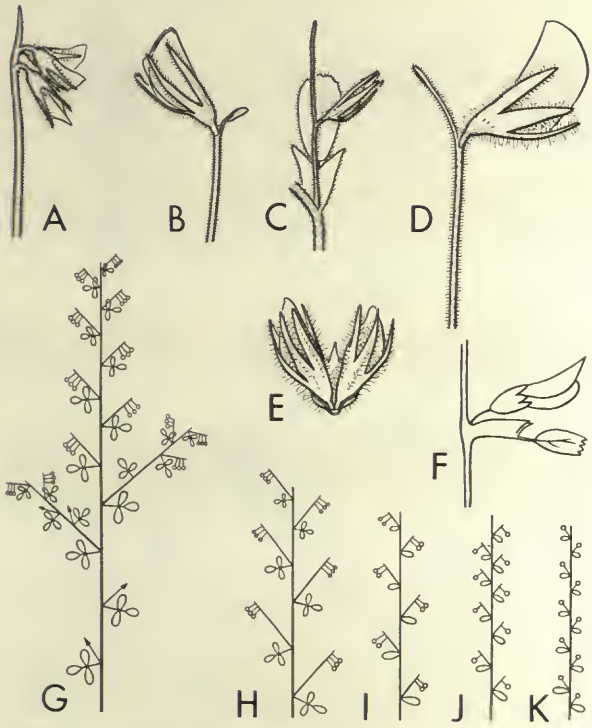

FIGURE 18. *Ononis rotundifolia*.—A. Two-flowered florescence.—B–D. Uniflorous florescences of *O. natrix* (B, D) and *O. breviflora* (C).—E, F. *O. arvensis*. Two-flowered sessile florescence from the adaxial side (E); uniflorous sessile florescence in the axil of the subtending leaf (F).—G. Diagram of a synflorescence of *O. rotundifolia*, with long and short paracladia.—H. Distal part of G, showing the zone of short paracladia in comparison to the same zone of *O. geminiflora* (I), *O. natrix* (J), *O. alopecuroides* (K). A–E, Original Troll (unpublished); F after Parkin (1914); G–I. after Waldorf (unpublished).

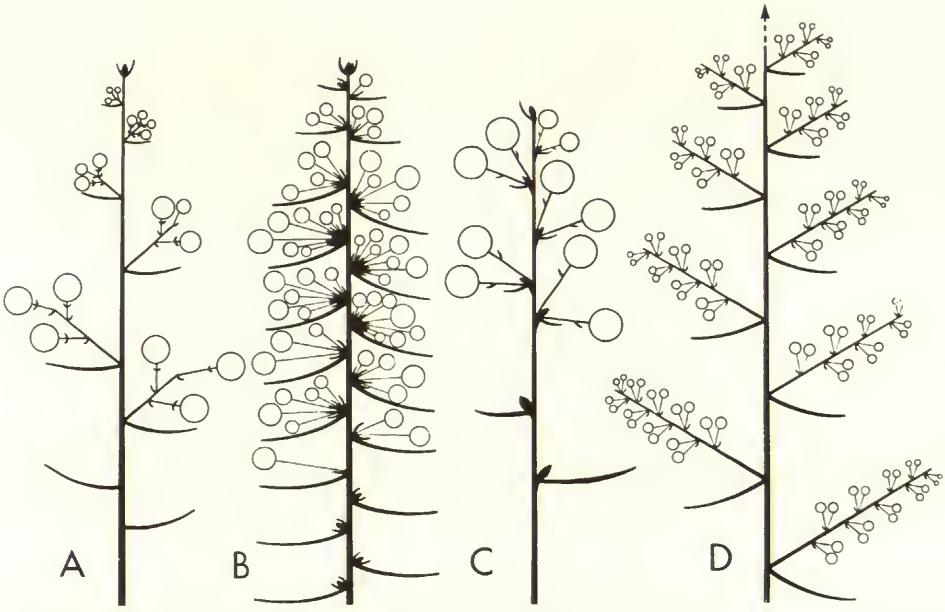

FIGURE 19. Diagrams of flowering shoots.—A. *Sutherlandia frutescens* (Herb. Web. 8904, Fernkloof, Hermanus, Cape region, South Africa).—B. *Psoralea pinnata* (Herb. Web. 8800, as A).—C. *Tephrosia grandiflora* (Aiton) Pers. (Herb. Web. 9034, near Grahamstown, South Africa).—D. *Lespedeza thunbergii* Nakai (Bot. Garden München); subtending bracts of the flowers and terminal buds omitted.

FIGURE 20. *Tephrosia grandiflora*, flowering shoot. Troll (unpublished).

FIGURE 21. *Laburnum anagyroides*, long shoot with unfolding flowering brachyblasts, with completely bracteose (A) and with frondose (B) leaves. Original Troll (unpublished).

FIGURE 22. *Laburnum anagyroides*, unfolding brachyblasts. Original Troll (unpublished).

FIGURE 23. *Laburnum anagyroides*, unfolding (A) and flowering bracteose (B) brachyblasts. Original Troll (unpublished).

FIGURE 24. A. *Gymnocladus dioicus,* flowering branch.—B. *Caragana arborescens;* piece of a long shoot with two unfolding short shoots bearing scars of cataphylls and rests of leaves and peduncles of the last season. Original Troll (unpublished).

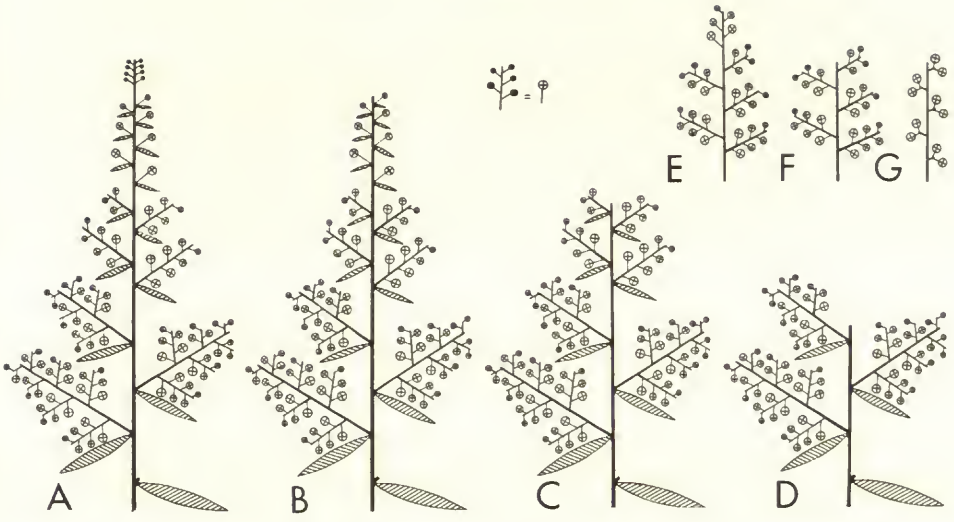

FIGURE 25. Derivation of various forms of synflorescences of the genus *Acacia*. Complete polytelic synflorescence (A) forming a heterothetic pleiobotryum, from which the most complete form of a synflorescence (B) existing within the genus *Acacia* can be derived by truncation.—C–G. Derivation of the inflorescences of the Racemosae (F) and Dicephalae (G). After Troll.

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REFERENCES

ARROYO, M. T. K. 1981. Breeding Systems and Pollination Biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

DUTAILLY, M. G. 1877. Observations organogéniques sur les inflorescences unilatérales des Légumineuses. Association Française pour l'Avancement des Sciences. Comptes rendus de la 5e sess., Clermont-Ferrand, 1876: 514–524, pl. VIII, IX.

GOEBEL, K. VON 1882. Ueber die Verzweigung dorsiventraler Sprosse. Arbeiten Bot. Inst. Würzburg 2: 353–436, Taf. VIII–XII.

_____. 1928. Organographie der Pflanzen. 3. Aufl. I: 405–411.

_____. 1931. Blütenbildung und Sproßgestaltung (Anthokladien und Infloreszenzen), II. Organographie der Pflanzen, Ergänzungsband 2: 230–235.

HOFMEISTER, W. 1868. Allgemeine Morphologie der Gewächse. *In:* W. Hofmeister (editor), Handb. physiologischen Botanik, Bd. 1, Abt. 2.

HOPKINS, M. C. 1984. Floral biology and pollination ecology of the Neotropical species of *Parkia*. J. Ecol. 72: 1–23.

IRMISCH, T. 1848. Botanische Notizen. Bot. Zeitung (Berlin) 6: 894–898.

_____. 1849. Ueber die Anordnung der Blüthenstände bei einigen Kleearten. Bot. Zeitung (Berlin) 7: 513–521.

———. 1851. Ueber die Blüthenstände einiger Leguminosen. Bot. Zeitung (Berlin) 9: 673–681, 689–697, pl. 10.

———. 1859. Ueber *Lathyrus tuberosus* und einige andere Papilionaceen. Bot. Zeitung (Berlin) 17: 57–63, 65–72, 77–84, Taf. III.

Köhler, M. A. 1923. Köhler's Medizinal-Pflanzen. 2. Aufl. (Hrsg. G. Pabst). M. Bernmühler, Berlin.

Neville, P. 1958. Contribution a l'étude de quelques types de rameaux de *Gleditschia triacanthos* L. Monspeliensia, sér. Botanique, fasc. 10.

Parkin, J. 1914. The evolution of the inflorescence. J. Linn. Soc., Bot. 42: 511–563.

Poncy, O. 1986. Le Genre *Inga* (Légumineuses, Mimosoideae) en Guyane Française. Systématique, Morphologie des Formes, Ecologie. "Studies on the Flora of the Guianas," no. 13. Mém. Mus. Natl. Hist. Nat. Sér. B, Bot. Tome 31: 13–15.

Rohrbach, P. 1870. Beiträge zur Morphologie der Leguminosen. Bot. Zeitung 28: 817–829, Taf. XIII.

Schneider, O. H. 1925. Zur Kenntnis dorsiventraler Blütenstände in der Familie der Papilionaceen. Bot. Arch. 11: 26–39.

Sell, Y. 1980. Physiological and phylogenetic significance of the direction of flowering in inflorescence complexes. Flora 169: 282–294.

Taubert, P. 1890. Monographie der Gattung *Stylosanthes*. Verh. Bot. Vereins Prov. Brandenburg 32: 1–34.

———. 1891. Leguminosae. *In:* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien. III(3): 70–385.

Trecul, A. 1854. Notes sur l'inflorescence unilatérale du *Trifolium lupinaster*. Bull. Soc. Bot. France 1: 125.

Troll, W. 1950. Über den Infloreszenzbegriff und seine Anwendung auf die blühende Region krautiger Pflanzen. Akad. Wiss. Abh. Math.-Naturwiss. Kl. 15.

———. 1958. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1957: 39–50.

———. 1960. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1959: 112–130.

———. 1961. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1960: 81–96.

———. 1962. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1961: 113–126.

———. 1964. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1963: 113–137.

———. 1965. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1964: 93–111.

———. 1964–1969. Die Infloreszenzen. Typologie und Stellung im Aufbau des Vegetationskörpers I (1964): II/1 (1969). Jena.

———. 1969. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1968: 88–109.

———. 1970. Bericht d. Komm. f. Biolog. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1969: 82–101.

———. 1973. Bericht d. Komm. f. Botan. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1972: 98–109.

———. 1974. Bericht d. Komm. f. Botan. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1973: 135–151.

———. 1975. Bericht. d. Komm. f. Botan. Forschg., Jahrb. Akad. Wiss. Lit. Mainz 1974: 128–142.

——— & H. Weber. 1955. Morphologie einschließlich Anatomie. Fortschr. Bot. (Berlin) 19: 18–27.

Vogel, S. 1968. Chiropterophilie in der neotropischen Flora. Neue Mitteilungen I. Flora 157: 562–602.

Weberling, F. 1983. Fundamental features of modern inflorescence morphology. Bothalia 14: 917–922.

Willkomm, M. & J. Lange. 1880. Prodromus Florae Hispanicae 3. Stuttgart.

Wydler, H. 1860. Kleinere Beiträge zur Kenntniss einheimischer Gewächse. Papilionaceae. Flora 1860: 17–32, 51–63, 83–96, 114 ff.

———. 1872. Kleinere Beiträge zur Kenntniss einheimischer Gewächse (Fortsetzung). Papilionaceae. Mitth. Naturf. Ges. in Bern 1871: 234–249.

EVOLUTIONARY IMPLICATIONS OF FLORAL ONTOGENY IN LEGUMES

Shirley C. Tucker[1]

Abstract

The three subfamilies of legumes are commonly distinguished on the bases of floral symmetry (radial in mimosoids, moderate zygomorphy in caesalpinioids, and moderate to strong zygomorphy in papilionoids), stamen number, and petal aestivation. Comparative floral ontogeny adds new character states to distinguish the subfamilies. In sepal initiation, mimosoids have either simultaneous whorled or helical order, caesalpinioids have helical order (rarely unidirectional), and papilionoids have unidirectional order of initiation. In petal and stamen initiation, mimosoids show simultaneous whorled order, while most caesalpinioids and papilionoids have unidirectional order of initiation. The three subfamilies are also distinguishable in early stages on the basis of petal position. Caesalpinioids and papilionoids have a median "standard" petal or vexillum on the upper or adaxial side, while mimosoids have the median petal on the lower or abaxial side of the flower. Mimosoids are radially symmetrical throughout development; most caesalpinioids become zygomorphic at petal initiation; and papilionoids are zygomorphic from sepal inception. Precocity of an advanced characteristic such as zygomorphy is itself considered specialized, so that earlier onset of zygomorphy among papilionoids would support this group being considered more advanced than caesalpinioids. On developmental bases, then, mimosoids stand somewhat isolated from the other subfamilies, having radial symmetry from inception, simultaneous or helical order of initiation, and an arrangement of organ sites basically different from the other two. Caesalpinioideae and Papilionoideae clearly are closely related to each other on developmental evidence, with papilionoids showing the more specialized features and absence of some of the less specialized ones. Tribal distinctions in Papilionoideae include free stamens versus different types of fusion among stamens. Developmentally, all papilionoids have originally free stamens. Possible types of fusion include diadelphy, monadelphy, and pseudomonadelphy. Ontogenetic evidence shows that pseudomonadelphy is derived from diadelphy. Most papilionoid tribes include some diadelphy; in addition, they usually also include either monadelphy or pseudomonadelphy, but not both. Since monadelphy and pseudomonadelphy represent a convergence, the evidence suggests that tribes tend to favor one pathway over the other. It also suggests that monadelphy has a high adaptive value. At the generic level, loss of certain organs (sepals, petals, or stamens) characterizes many legume genera. This "loss" usually results from suppression after initiation, but rarely are organs completely absent. In flowers with suppression of certain organs, there is no disruption of subsequent initiation or architecture of the flowers. Suppression may occur at different stages of development in different taxa. Where there is actual loss of organs, subsequent development is strongly disrupted in the form of atypical order of initiation, atypical organ sites, and extra organs formed. Complete loss of organs may have been a mechanism for macroevolution, since it affects many aspects of subsequent development and mature form.

I. Introduction

Because floral structural differences such as symmetry and petal aestivation effectively separate the three subfamilies of Leguminosae, the developmental bases for these features can be compared in order to determine the divergent developmental patterns responsible for the separation. Flowers are useful in that they are determinate in their development (unlike the vegetative plant body) and hence the complete process of development can be compared among taxa as in animal embryogeny. Comparative ontogeny of flowers had not been done critically until recently, when scanning electron microscopy (SEM) vastly improved resolution and accuracy of such studies. SEM is also

[1]Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803, U.S.A.

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relatively quick, compared to laborious reconstructions of sectioned buds, although such reconstructions often are needed to supplement SEM work.

As examples of comparative ontogenetic studies among legume flowers, this paper will discuss ontogenetic distinctions among subfamilies ("new" character states), intertribal distinctions in papilionoid legumes, and the relative consequences of suppression versus loss of organs.

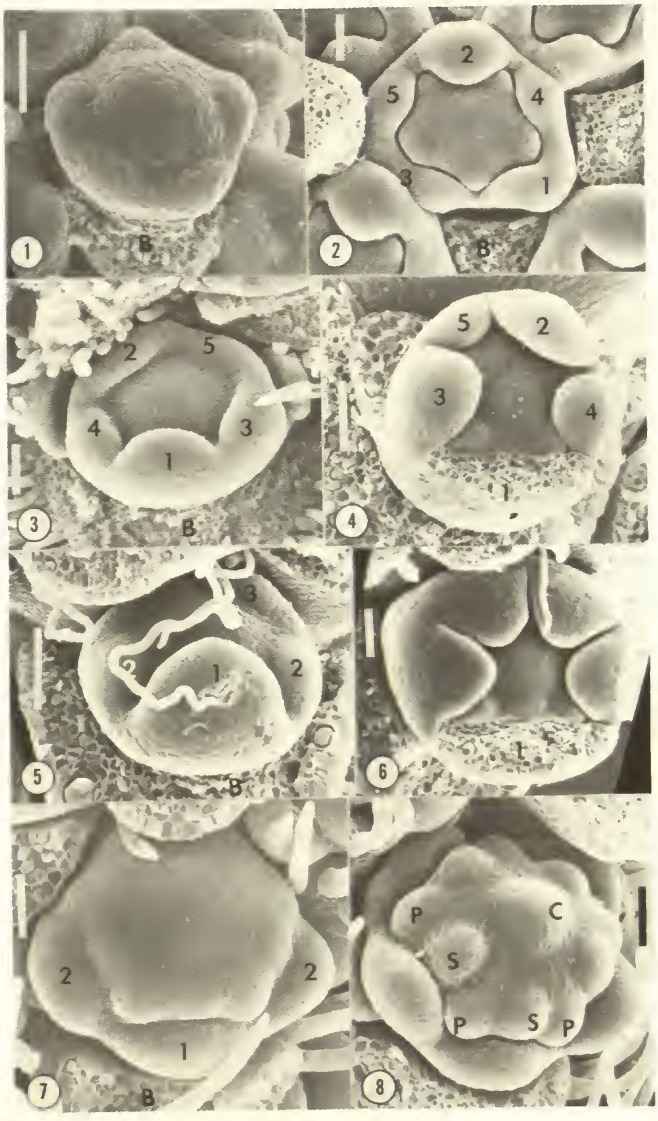
II. Ontogenetic Distinctions Among Subfamilies: "New" Character States

The order of initiation among organs in each whorl of floral organs differs among subfamilies of legumes (Table 1). In the radially symmetrical flowers of mimosoids (Gemmeke, 1982) simultaneous or synchronous initiation of organs in each floral whorl has been reported. Observations in my laboratory (Derstine & Tucker, 1985; Derstine & Ramírez-Domenech, 1985; Ramírez-Domenech & Tucker, 1988; Tucker, 1988a) have revealed that some mimosoids have simultaneous initiation (Fig. 1), others have helical initiation of sepals (Fig. 2). Study of the systematic distribution of helical versus whorled calyces among members of mimosoid tribes is currently under way. This example of convergent pathways of evolution illustrates a useful qualitative character only recognizable through ontogenetic study. The helical pattern is common to some taxa in both Caesalpinioideae and Mimosoideae, and would therefore seem to be the primitive condition. Generally, helical arrangements are considered to indicate primitiveness in other families such as the Magnoliaceae and Ranunculaceae, in both of which the whorled condition is derived. The sepals of most caesalpinioid taxa are initiated helically (Figs. 3, 4) but in *Caesalpinia cassioides* and *C. pulcherrima* they are unidirectional (Figs. 5, 6; Tucker et al., 1985). In papilionoids, sepals arise unidirectionally (Figs. 7, 8; Tucker, 1984b, 1987b). Older flower buds and flowers do not reveal whether the calyx was whorled or helical at initiation.

Another result of ontogenetic comparisons is the distinction in organ sites between mimosoids and the other two subfamilies (Table 1; Fig. 9). The median petal of a pentamerous mimosoid flower is abaxial (on the side of the flower adjacent to the subtending bract), while that of caesalpinioid and papilionoid flowers is adaxial (on the side remote from the subtending bract). As a corollary, the median sepal of a mimosoid flower is adaxial, while that of the other two subfamilies is abaxial. These differences were not illustrated by Eichler (1878) nor by Taubert (1894), who showed only tetramerous mimosoid flowers. These may have either two median petals (abaxial and adaxial) or none in the median sagittal plane (Ramírez-Domenech & Tucker, submitted). The difference in site of sepals and petals in pentamerous flowers is consistent between the subfamilies concerned in the 160 or more taxa we have examined so far. It should be emphasized that the determination of petal and sepal sites must be made early, during organogeny, because the pedicel

Table 1. Ontogenetic characteristics of legume subfamilies.

	Mimosoideae	Caesalpinioideae	Papilionoideae
Order of initiation of sepals	simultaneous or helical	helical, or unidirectional (rare)	unidirectional
Order of initiation of organs in corolla and stamen whorls	simultaneous	helical (rare) or unidirectional	unidirectional
Position of organs	median petal abaxial	median petal adaxial	median petal adaxial
	median sepal adaxial	median sepal abaxial	median sepal abaxial
Stage when zygomorphy becomes evident	rare; mid-stage during stamen enlargement	at petal initiation in most	at sepal initiation



FIGURES 1–8. Order of sepal initiation in the three legume subfamilies (SEM micrographs). Abaxial side and subtending bract (B) are at the base in each figure. 1, 2. Mimosoideae. —1. Simultaneous initation of five sepals in *Neptunia pubescens*. —2. Helically initiated sepals in *Leucaena leucocephala*. Petals also are present. 3–6. Caesalpinioideae. —3,4. Helically initiated sepals in *Bauhinia fassoglensis* (Fig. 3) and slightly older helically arranged sepals in *Cercis canadensis* (Fig. 4). The first and largest sepal (1) has been removed to reveal young petals. —5,6. Unidirectional sepal initiation in *Caesalpinia pulcherrima* just after initiation of the last two adaxial sepals (Fig. 5) and after equalization in size of four of the sepals (Fig. 6). Sepals are numbered in order of initiation in Figure 5. The largest, first sepal (1) has been removed in Figures 6, 7, 8. Papilionoideae: *Genista tinctorum*. —7. Unidirectional sepal initiation; the single abaxial sepal (1) was initiated first, followed by the two lateral ones (2). The two adaxial sepals are not yet evident. —8. The five sepals (unlabeled) are all present and equal in size. Petals (P), outer stamens (S) and carpel (C) are also present; not all are labeled. Scale bars = 50 μm. Selected figures reprinted with permission from American Journal of Botany (1, 3, 5, 7, 8,) and from Royal Botanic Gardens, Kew (2–5).

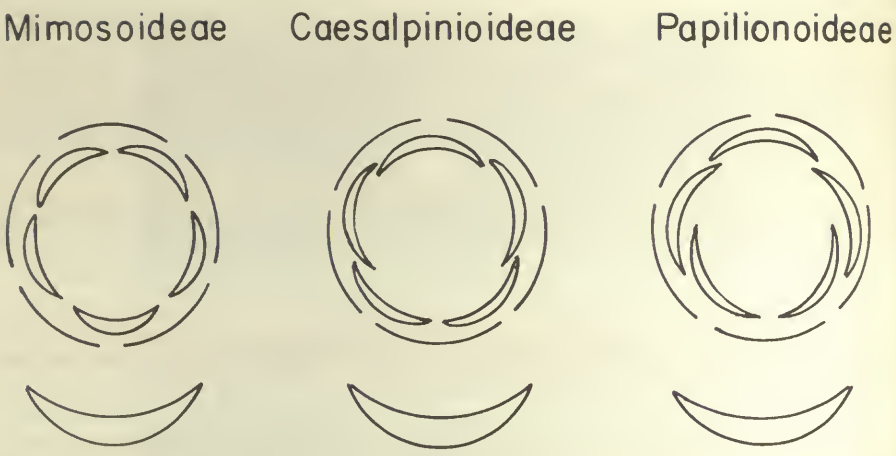


FIGURE 9. Floral diagrams of the three legume subfamilies to show typical positions of sepals and petals, and petal aestivation.

distal to the subtending bract may twist as it grows. As a result, the positional relationship between bract, sepal, and petal changes or is not determinable later on.

Zygomorphy is first established at petal initiation in Caesalpinioideae and at sepal initiation in Papilionoideae (Tucker, 1984b). The only mimosoid example of dorsiventral or zygomorphic symmetry is in the male flower of *Neptunia pubescens* (Tucker, 1988a). The dorsiventral condition is manifested much later in ontogeny than in flowers of the other two subfamilies. In *N. pubescens* (Tucker, 1988a), the ten stamens are alike up to a height of 150 μm in mid-development. Thereafter, the five more abaxial stamen primordia are suppressed and do not develop anthers or microsporocytes. The five more adaxial stamen primordia develop anthers and microsporocytes (the latter, however, are fewer in number than in the anthers of perfect flowers of the same species). This example of zygomorphy in the subfamily is interesting in that it results from divergent pathways of development at a much later stage of ontogeny than in the subfamilies where zygomorphy is a well-established and stable feature determined very early in development.

Gould (1977) emphasized that the timing at which a character is expressed during development is itself subject to genetic control and can change during evolution. Precocity of a characteristic is a specialization. Stebbins (1974) discussed the increasing precocity of fusion among organs as an example of specialization. The earlier in development that parts become fused, the more advanced the condition. In legumes the usual order of organ initiation is: sepals, petals, stamens, and carpel. In the timing of expression of zygomorphy, the papilionoids are most specialized, since the condition is expressed at sepal initiation. Most caesalpinioids show a less specialized condition, since zygomorphy is expressed first at petal initiation, after sepal initiation. In the mimosoid *Neptunia*, the condition is manifested well after initiation of all floral organs at mid-development (Tucker, 1988a). Hence, the condition in the mimosoid subfamily would be considered the least advanced state of zygomorphy of the three. In other families there are even later expressions of zygomorphy during development: zygomorphy in *Aconitum* and *Delphinium* (Ranunculaceae) becomes evident only at organ expansion: the adaxial petals enlarge more than the abaxial ones (Mair, 1977).

III. Intertribal Distinctions in Papilionoid Legumes

The characterization of papilionoid tribes has become far better defined with the establishment of more and smaller tribes, and with the recent flood of new types of information about legumes

(Polhill & Raven, 1981). One intriguing developmental difference that has come to light is a generic distinction between taxa exhibiting two types of monadelphous androecium. In all legumes studied, representing all subfamilies and most tribes, the stamens are free in young stages (Tucker, 1984b, 1987b; Tucker et al., 1985).

Fusion can occur among the stamens in different combinations, but the common patterns are monadelphy (all ten stamens raised up by intercalary or zonal growth below their bases) or diadelphy (intercalary or zonal growth below nine stamens, leaving the vexillary stamen free). In at least 54 genera in nine tribes a condition called pseudomonadelphy is found (Table 2, based primarily on Hutchinson, 1964). The term "pseudomonadelphy" was used by Gillett (1961) and by Sousa & Sousa (1981); the latter held the opinion that pseudomonadelphy in Lonchocarpinae was a less specialized condition than diadelphy. In *Erythrina caffra*, a pseudomonadelphous species, nine of the initially free stamen primordia (Fig. 11) fuse basally by intercalary growth, yielding a diadelphous condition (Fig. 12) in buds. Just before anthesis, the filament of the free vexillary stamen becomes fused laterally to the adjacent sides of the fused staminal tube, so that all ten are fused at anthesis (Fig. 13). Pseudomonadelphous androecial tubes are recognizable by the fenestrations (fenestrae) basally on either side of the vexillary stamen (Fig. 13), although rarely they are absent as in *Disynstemon* (Geesink, 1984). Gillett (1961) discovered that in ten species of *Millettia* the vexillary stamen is free (diadelphous) in young buds but fused in older ones or in mature flowers. He used the term "pseudomonadelphous" for this condition and suggested that it characterizes the genus. The developmental relationship between pseudomonadelphy and diadelphy is demonstrated by studying late stages of buds. Frequently, both diadelphy and pseudomonadelphy occur (in 22 of the 54 genera, Table 2) as alternate conditions in the species of these genera. Conversely, it is rare to find genera (3 of the 54) in which both monadelphy and pseudomonadelphy occur; *Aeschynomene*, *Desmodium*, and *Erythrina* are said to include species showing one or the other condition, or diadelphy. Tribal distribution of pseudomonadelphy versus monadelphy is less clear-cut; monadelphy is found in 18 papilionoid tribes (Table 3), while pseudomonadelphy occurs in 10 with overlap reported in 3 (Table 2). The pseudomonadelphous condition (Tucker, 1987a) seems clearly a specialization derived from the diadelphous condition (Fig. 18), and it appears to have arisen in at least nine tribes in parallel. Genera have tended to develop either monadelphy or pseudomonadelphy, but not both. Whether the two are

TABLE 2. Occurrence of pseudomonadelphy in papilionoid tribes (arranged according to Polhill & Raven, 1981; data primarily from Hutchinson, 1964).

Millettieae:	*Afgekia*, *Apurimacia*, *Chadsia*, *Craibea*[d], *Derris*[d], *Hesperothamnus*, *Lonchocarpus*, *Millettia*[d], *Muellera*, *Mundulea*, *Paratephrosia*, *Piscidia*, *Pongamia*, *Sylitria*, *Tephrosia*, *Willardia*, *Wisteria*[d]
Robinieae:	*Coursetia*[d], *Diphysa*, *Lennea*, *Robinia*
Desmodieae:	Desmodiinae: *Desmodium*[m,d], *Droogmansia*
Phaseoleae:	Phaseolinae: *Oxyrhynchus*, *Psophocarpus* Clitoriinae: *Centrosema*[d], *Clitoria*[d], *Periandra*[d] Erythrininae: *Erythrina*[m,d] *Rhodopis*[d] Diocleinae: *Camptosema*[d], *Canavalia*, *Cleobulia*, *Collaea*, *Cratylia*, *Dioclea*, *Macropsychanthus* Glycininae: *Pueraria*[d]
Psoraleeae:	*Hoita*[d], *Orbexilum*[d], *Otholobium*, *Pediomelum*[d], *Psoralea*[d]
Aeschynomeneae:	*Aeschynomene*[m,d]
Hedysareae:	*Ebenus*, *Eversmannia*[d], *Onobrychis*, *Stracheya*, *Taverniera*
Vicieae:	*Lathyrus*[d], *Pisum*[d], *Vicia*[d]
Trifolieae:	*Trifolium*[d], *Trigonella*[d]

Key: m, monadelphy also present; d, diadelphy also present.

W
K
10
S
P
P
S
S
P
11
S
12
S
13
2
3
4
1
14
S
2
P
3
P
4
15
16
P
P
17

TABLE 3. Occurrence of types of androecial fusion among papilionoid tribes.

Abreae	M			Genisteae	M		
Aeschynomeneae	M	D	P	Hedysareae		D	P
Amorpheae	M			Indigofereae	M	D	
Bossiaeeae	(M)	D		Liparieae	M	D	
Brongniartieae	M	D		Loteae	M	D	
Carmichaelieae		D		Millettieae	M	D	P
Cicereae		D		Phaseoleae	M	D	P
Crotalarieae	M	(D)	(P)	Psoraleeae	M	D	P
Dalbergieae	M	D	P	Robinieae		D	P
Desmodieae	M	D	P	Trifolieae	M	D	P
Euchresteae		D		Vicieae	M	D	P
Galegeae	M	D					

Key: M, monodelphy; D, diadelphy; P, pseudomonadelphy; () , rare.

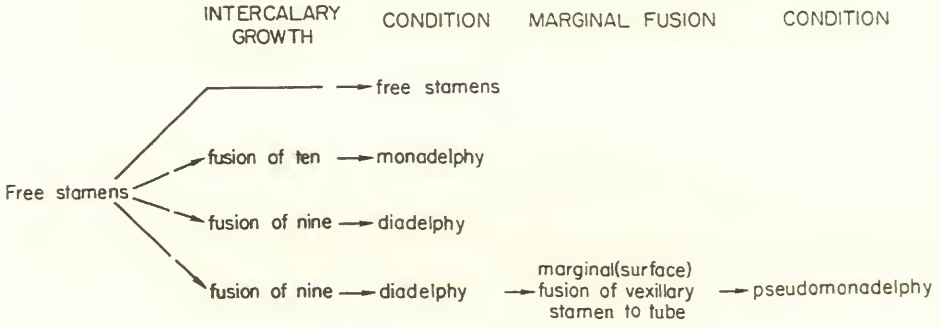


FIGURE 18. Expression of androecium in papilionoid tribes.

functionally equivalent is not known, but in practice (as indicated by some instances of lack of recognition of the pseudomonadelphous condition in taxonomic descriptions) the two are easy to confuse. Monadelphy and pseudomonadelphy probably can be considered instances of convergent evolution; pseudomonadelphy is "added on" to the diadelphous condition during late ontogeny (Fig. 18).

Diadelphy and monadelphy both result from one fusion process (intercalary or zonal growth), while pseudomonadelphy results from intercalary growth plus an additional and different process at

← FIGURES 10–17. Suppression of organs, and fusion of the androecium (SEM micrographs). —10. Flower of *Amorpha fruticosa*. The wing (W) and keel (K) petals are visible on one side. All five petals are initiated, and all but the standard (not shown) are suppressed. 11–13. Androecial fusion in *Erythrina caffra*. —11. Stamens (S) are free directly after they are initiated; petal (P), carpel (C). —12. Filaments of nine stamens have become fused basally (at arrow) by zonal growth below their level of attachment. The tenth stamen (S) is free. —13. The tenth stamen (S) has become fused to the adjacent sides of the filament tube above the free base to produce a pseudomonadelphous androecium. 14–17. Initiation and suppression in *Schotia brachypetala*. Helical initiation of four sepals numbered consecutively. —15. A fifth sepal has been initiated; all are numbered in consecutive order. The petals (P, only two labeled) are starting to initiate. —16. Four sepal scars are evident; the adaxial (at top) is composed of two sepals fused basally. The five petals are indicated by arrows; one abaxial one (at lower right) had been suppressed directly after initiation. All floral organs are present. —17. Lateral view of flower showing one rudimentary suppressed petal primordium (at arrow); other petals (P) are enlarging. Scale bars = 50 μm in Figures 10, 11, 14, 15; = 500 μm in Figures 12, 13, 16, 17. Figures 12–14, 16 reprinted with permission from Royal Botanic Gardens, Kew.

a later stage of development: surface fusion between sides of the filaments concerned (Fig. 13). In genera where it is reported, fusion does not include the base of the vexillary stamen, which remains free, forming fenestrae. If there are instances of pseudomonadelphy that include the entire length of the vexillary filament, these would only be recognizable by ontogenetic study; none are known so far.

IV. Suppression Versus Loss of Organs

Loss of certain organs (sepals, petals, or stamens) typifies some taxa of legumes. The examples discussed herein include flowers with missing sepals, petals, or stamens in species of *Amorpha* and *Ateleia* (Papilionoideae), *Bauhinia, Saraca,* and *Schotia* (Caesalpinioideae). Most show varying degrees of organ suppression; *Ateleia* and *Saraca* show loss of organs.

Petal Suppression in *Amorpha*

The flower of *Amorpha fruticosa* (Tribe Amorpheae) has only a standard (vexillary) petal at anthesis. Examination of young buds (Fig. 10), however, shows that all five petals are initiated but that the wing and keel petals are suppressed after initiation and stop enlarging at a height of about 50–60 μm. Two whorls of five stamens each are next initiated in succession, the members of each whorl arising in unidirectional order from the abaxial side as in most papilionoids studied (Tucker, 1984a, b, 1987b). Stamen positions, number, and order of initiation all conform to the usual pattern for papilionoid flowers.

Sepal and Petal Suppression in *Schotia*

Each flower of *Schotia brachypetala* (Caesalpinioideae: Detarieae) regularly has four sepals and four (rarely five) petals. Studies of young buds (Tucker, 1988b) reveal that five sepals are initiated in the usual sites and in the usual helical order (Fig. 14). The last-initiated sepal, on the adaxial side of the floral apex, remains small (Fig. 15) and is later not noticeable at anthesis. Five petals are initiated in the normal sites and order (Fig. 15), although subsequent development is atypical. One keel petal (on the abaxial side), one of the first two initiated, is suppressed shortly after initiation (Fig. 16) and forms only a small rudiment while other petals enlarge (Fig. 17). The organs suppressed in the sepal and petal whorls are on opposite sides of the apex and differ also in whether the suppressed organ is initiated early or late in its whorl. Members of the two whorls of stamens are positioned in the expected sites, and they are initiated unidirectionally in each whorl starting from the abaxial side. The suppression of a sepal and a petal in *Schotia brachypetala* does not disturb subsequent organogeny or symmetry. According to Codd (1956), *S. brachypetala* may have some or all petals reduced in number or in size. Other species of *Schotia* all have four (rarely five) sepals and five full-sized petals.

Stamen Suppression in *Bauhinia*

Suppression of organs after their initiation has undoubtedly been important in the evolution of legumes. Several other examples of petal and stamen suppression have been studied, particularly in species of *Bauhinia* (Tucker, 1984b, 1987b). Suppression of certain organs (petals, stamens, and/or carpel) in different species of *Bauhinia* has set the stage for dioecy, heterochrony, and transference of organ function, as well as a wide range in stamen number and expression per species. But as in

other examples of suppressed organs, there is no disruption of the usual positions of subsequent organs, nor of their usual order of initiation. The number of stamens has increased over the usual number of ten in some species of *Bauhinia,* in species showing stamen suppression at some point in development. There are species of *Bauhinia* having one, two, three, five, seven, eight, nine, or ten fertile stamens per flower; species also vary in positions of fertile and sterile stamens or staminodia (Urban, 1885). Two examples are discussed below, from more detailed studies elsewhere (Tucker, 1988c).

In *Bauhinia divaricata,* each flower has one functional stamen and four (or rarely five) petals; the standard is rarely more than a rudiment. All five petals are initiated; the wing petals elongate first, the keel petals later and irregularly. All ten stamens are initiated in the usual positions and usual order of initiation; often there are extra stamen primordia (12–13). All but the vexillary stamen are suppressed early (Fig. 19) and do not form anthers or pollen. The keel petals enlarge first, with wings delayed (Fig. 19); wings start to enlarge later (Fig. 20). Just before anthesis, the nine or more suppressed stamen primordia undergo zonal growth and form a staminodial tube around the stipe of the carpel (Fig. 21). As this flower is bat-pollinated (Percival, 1974), the staminodial tube may offer some protection or support for the carpel.

In other species, suppression after initiation leads to dioecy. *Bauhinia malabarica* trees bear only male, female, or hermaphrodite flowers. All organs are present in each morph, and flowers are similar at mid-development (Figs. 22, 23). One set of sexual organs is suppressed thereafter in the female flower and remains rudimentary (Fig. 24, female flower). A perfect or hermaphrodite flower, minus petals and sepals, is shown in Figure 25.

Variable suppression has functioned in speciation in *Bauhinia,* accompanied by changes in inflorescence type, receptacle characters, sepal morphology, stipe connation, style and stigma structure, and various vegetative features (de Wit, 1956). De Wit used stamen number as one basis to segregate several genera within *Bauhinia,* although these are not universally accepted. He said that *Bauhinia* s.l. has the morphological latitude of a tribe; the segregate genera are not sharply distinguished, however.

SUPPRESSION OF POLLEN FORMATION IN *ATELEIA*

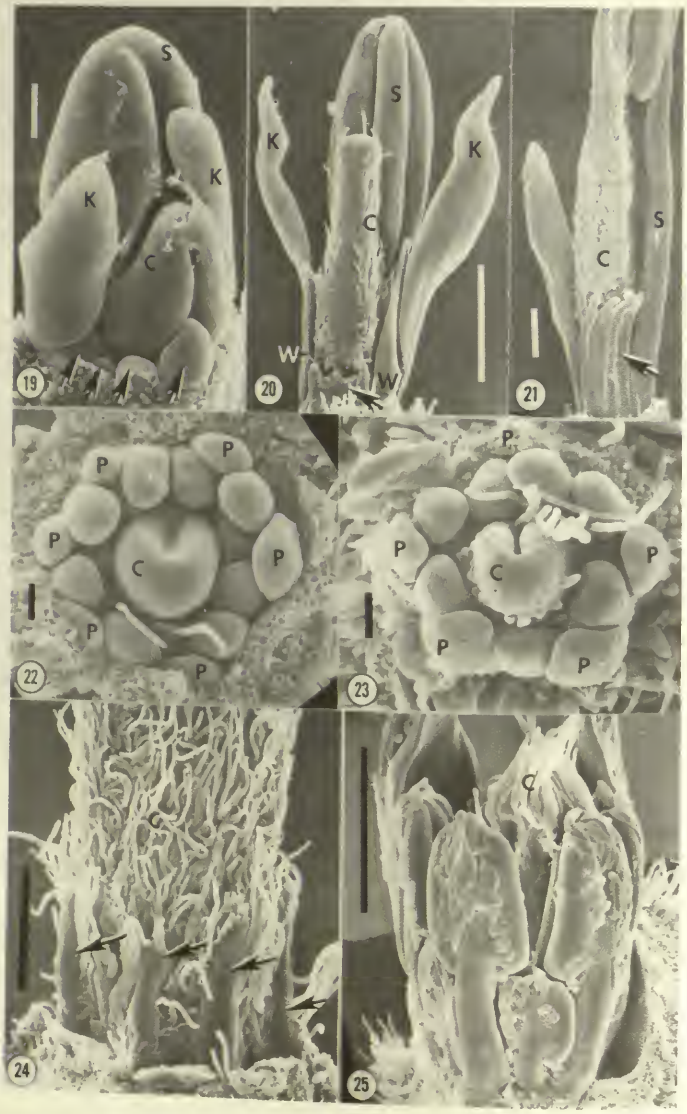
Suppression can occur quite late in floral development, as in the expression of female or male flowers in *Ateleia herbert-smithii* (Papilionoideae: Sophoreae). These are very similar in appearance (Figs. 30, 31) and differ only in that pollen does not form in the anthers of the functionally female flowers and that the carpel remains small and sterile in the male flowers (Tucker, 1988b).

CLEISTOGAMOUS SUPPRESSION OF ORGANS

Cleistogamy is another common example of suppression of organs as opposed to loss. Several examples are known in the Leguminosae, such as *Amphicarpaea, Argyrolobium, Glycine, Lathyrus, Lespedeza, Ononis, Robinia, Tephrosia, Vicia,* and *Voandzeia* (Lord, 1981; Uphof, 1938). Ten species of *Lespedeza* were compared by Hanson & Cope (1955). Usually the petals are all suppressed after initiation in cleistogamous flowers, although the positions and numbers of organs are unchanged from chasmogamous flowers of the same species. The order of initiation has not been compared between cleistogamous and chasmogamous members of any legume.

LOSS OF PETALS IN *ATELEIA*

Examples of true loss of organs are not nearly so common among legumes as examples of suppression (Tucker, 1987b). Each flower of *Ateleia herbert-smithii* (Papilionoideae: Sophoreae)



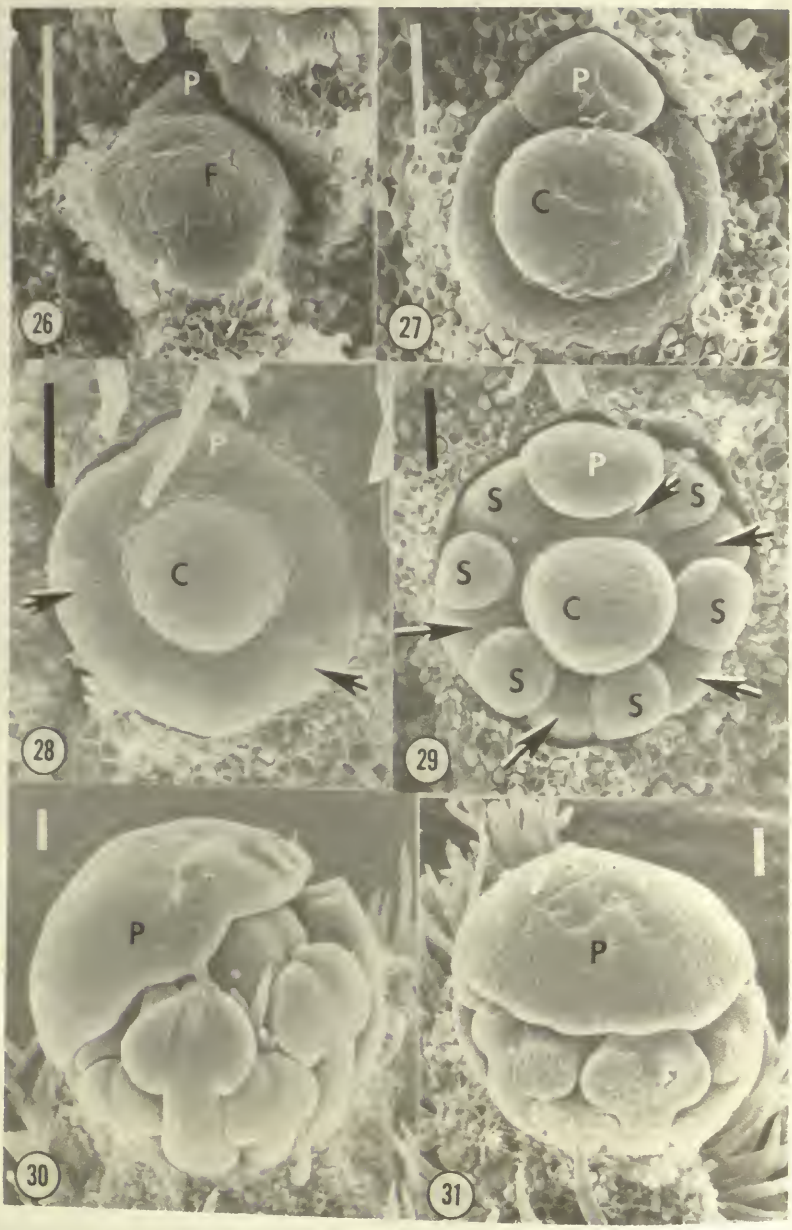
FIGURES 19–25. Suppression of organs after initiation in species of *Bauhinia* (SEM micrographs). 19–21. *Bauhinia divaricata*. —19. Flower with one large stamen (S), two keel petals (K), a carpel (C), and several suppressed petals and stamen primordia (at arrows). —20. Later stage in which the wing petals (W) are starting to enlarge. The suppressed stamen primordia (at arrow) are starting to heighten by zonal growth. —21. Older stage with the single fertile stamen (S) at right. The suppressed stamen primordia have elongated to form a staminodial tube (at arrow) around the carpel base (C). 22–25. Female (Figs. 22, 24) and perfect (Figs. 23, 25) flowers of *Bauhinia malabarica*. Sepals and petals have been removed. —22, 23. Polar view of female and perfect flowers at mid-stage before organ differentiation, when the two types are very similar. Both tend to have extra stamens beyond the usual ten; petals (P), carpel (C), stamens are not labeled. —24. Female flower. Stamen primordia (at arrows) have become suppressed at mid-stage and are tiny hairy projections, lacking anthers. —25. Perfect flower with stamens of two sizes and carpel (C). Scale bars = 50 μm in Figures 19, 22, 23; = 500 μm in Figures 20, 21, 24, 25. Figures 19 and 20 reprinted with permission from Academic Press, Figures 20, 23, 24 from Royal Botanic Gardens, Kew, and American Journal of Botany, and Figure 24 from E. Schweizbart'sche Verlagsbuchhandlung.

has only one petal. Examination of organogenetic stages of buds (reported more fully in Tucker, 1988b) reveals that only the vexillary petal is initiated. In other papilionoids, the vexillary petal is the last of the five petals to be initiated, after two abaxial keel petals and two lateral wing petals (Tucker, 1984a, b, 1987b). Subsequent organogeny of stamens in *Ateleia* is different from the pattern found in most other legumes, in which members of each of the two whorls of stamens would be initiated in unidirectional succession from the abaxial side of the floral apex. Male and female flowers have very similar organogeny. After the calyx and vexillary petal are initiated (Fig. 26), a meristematic ring forms (Fig. 27), from which ten stamens or more are initiated (Fig. 29). The order of stamen initiation is neither unidirectional, helical, nor whorled. The first two stamen primordia form laterally at the 4 and 8 o'clock positions (Fig. 28); by position they belong to the antesepalous or outer whorl. The next stamens are the antesepalous pair at the 10 and 2 o'clock positions. Order of the remaining six stamens is somewhat variable. Commonly, the three most abaxial stamens (usually the earliest to initiate) are the last-formed. The total number of stamens is 8–10(–13, 14) according to Janzen (this volume). With abnormal numbers above or below ten, the positions of stamens are also atypical. Older flowers show two whorls of five stamens each (Fig. 29). The stamen number in other species of *Ateleia* varies from 6 to 10(–11). Rudd (1968), in the genus description, reported occasionally varying stamen numbers in flowers of the same inflorescence. Another aspect of stamen development is profoundly affected in this species; no pollen is produced in stamens on some trees, which then function as female only. Male and female flowers (Figs. 30, 31) are still very similar at anthesis. *Ateleia herbert-smithii* is the only species in its genus (of 16 or so species) that is reported to be functionally dioecious (Janzen, 1981; see Janzen, this volume, for further details on biology).

Because the single petal in *Ateleia* does not allow one to determine petal aestivation, Rudd (1968) indicated that there is some uncertainty about whether *Ateleia* belongs in Papilionoideae or Caesalpinioideae. She stated that the peltate stigma and the occasional increase in stamen number over ten suggest a possible affinity to Caesalpinioideae. *Ateleia* is currently placed in the tribe Sophoreae of Papilionoideae on the basis of the ten free stamens per flower; however, the Sophoreae are suspected to be heterogeneous. Ontogenetic evidence shows that all legume stamens are free at the start of floral development and that varying pathways then bring about diadelphy, monadelphy, or a continued free condition (Tucker, 1987b). Hence, the free condition among stamens is the least modified pathway and could prevail in any legume simply by failure of subsequent fusion. Therefore the tribe Sophoreae, based primarily on free stamens, is likely to include widely diverse elements; other criteria besides free stamens are needed (Janzen, this volume, provides other arguments). Some support for transferring *Ateleia* to caesalpinioids may be forthcoming from ontogenetic comparisons, since most of the few other known examples of missing organs are all in Caesalpinioideae, except the anomalous tribe Swartzieae and an occasional missing vexillary stamen in several other papilionoids.

LOSS OF A STAMEN WHORL AND TRANSFORMATION OF PETALS TO STAMENS IN *SARACA*

A caesalpinioid example of early and extreme suppression of organs as well as of loss of parts is *Saraca* (tribe Detarieae). The flower of *Saraca declinata* has four large showy sepals, no petals, four stamens, and a carpel. Other species of *Saraca* are similar except for stamen number. For example, developmental studies of *S. declinata* (Tucker, 1988b) reveal that all is not what it seems at anthesis; there are five, not four sepals, but two become fused by basal zonal growth so that they appear as one. Five primordia are initiated in the usual petal sites alternating with sepal sites (Fig. 32), but none become petals. Four of them differentiate as stamens (Fig. 39). The fifth (in the vexillary position) remains rudimentary (Figs. 36, 38) and the flower appears to lack petals. Three primordia are initiated abaxially and laterally in the outer stamen whorl (Figs. 33, 35). These remain rudimentary (Fig. 38), however, and later become swellings on the basal collar of the



FIGURES 26–31. Floral organogenesis and organ loss in *Ateleia herbert-smithii* (SEM micrographs). Sepals have been removed in all. Abaxial side is at base of figure in Figures 26–29. —26. A solitary petal, the standard (P), has been initiated on the adaxial side of the floral apex (F). —27. Stamen ring has formed at periphery of floral apex. The central mound is the carpel primordium (C). —28. Distinct stamen primordia are forming within ring at arrows; standard petal (P), carpel (C). —29. Six stamens of outer whorl (one extra) are labeled S, and five stamens of inner whorl are indicated by arrows. —30, 31. Male flower and female flower respectively, showing solitary standard petal (P) and dimorphic stamens. Scale bars = 50 μm. Figures 26 and 27 reprinted with permission from E. Schweizbart'sche Verlagsbuchhandlung, and Figures 27, 30, 31 from Royal Botanic Gardens, Kew.

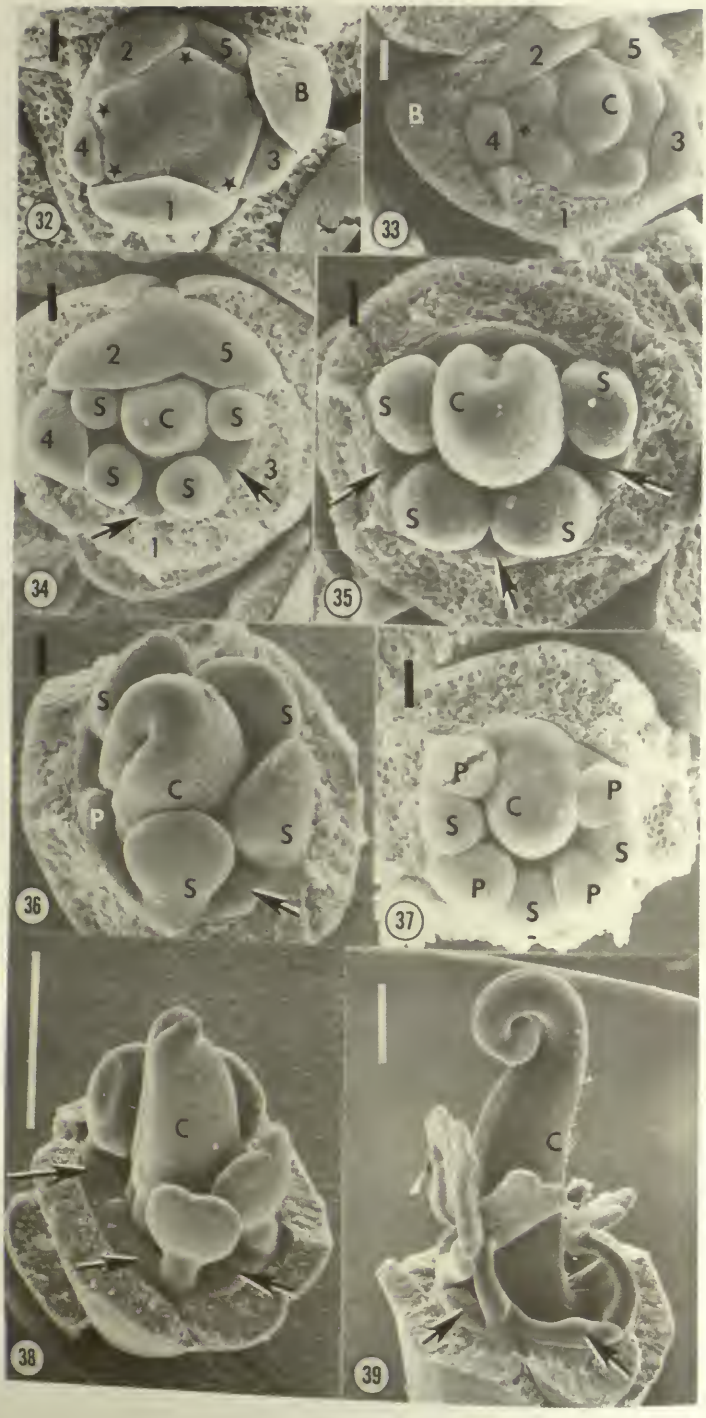
stamens (Fig. 39). The inner whorl of stamens is completely absent in all species. In *S. indica* the seven stamens (Fig. 37) result from four converted petal primordia (excepting the vexillary member) and the single abaxial and two lateral stamen primordia of the outer stamen whorl. The stamens and vexillary petal on the adaxial side of the flower are the organs most completely suppressed in *Saraca*. Carpels are suppressed after initiation in many of the flowers as well, so that a high proportion of flowers in any inflorescence is functionally male (Zuijderhoudt, 1968). Zuijderhoudt gave the number of stamens in *S. indica* as (5–)6–8(–10), so that presumably the missing stamens on the adaxial side do form in some flowers. Thompson (1924) mentioned that in *Saraca triandra*, also apetalous, he found one young bud with five slightly larger primordia which he thought were petals. These were represented in tiny drawings of sections. He never found petals in larger buds.

Suppression or loss of some sepals or petals (the earliest-formed organs) in *Ateleia* and *Saraca* results in profound disruption of subsequent ontogeny, particularly of events determining stamen number and order of initiation, and symmetry. These changes are of such major magnitude as to suggest that loss of organs may have been a mechanism of macromutation which could create new genera. Hilu (1983) discussed numerous monogenic mutations of flowers, including loss of parts, which he believed are macromutations responsible for abrupt evolutionary change in various plant families. Neither *Ateleia* nor *Saraca* is said to have closely related genera; each genus includes a closely related group of species. The evidence suggests that both could have arisen by an abrupt change. Complete loss of one or more early organs such as petals could divert ontogeny into alternate developmental pathways in which the factors determining symmetry, position and number of organs, and their order of initiation are subject to major changes.

Many caesalpinioid taxa in the tribes Cassieae, Amherstieae, and Detarieae have reduced numbers of floral organs (Thompson, 1924, 1925). *Dialium* and *Dicorynia*, for example, have only two stamens per flower, as well as reduced numbers of petals. Thompson (1924, 1925) studied sectioned buds and open flowers of many of these and showed the positions of different organs. He did not recognize that members of each whorl are not simultaneous. In Amherstieae (Thompson, 1924), he showed that flowers are commonly four-merous in the calyx; the missing sepal is one of the adaxial and last-formed organs. Petals in these taxa are either completely absent or the keel petals (and sometimes also wing petals) are missing. The keel petals are the first ones of their whorl to be initiated, the wings next, and the standard is the last initiated. The standard petal tends to persist if any petals are present; it is usually showy and forms a five-merous perianth together with the four sepals, which also tend to be showy here. In the tribe Cassieae (Thompson, 1925), flowers tend to lose an adaxial sepal (as in *Labichea*), the abaxial keel petals, and sometimes wing petals as well. Missing stamens may be on either the abaxial (*Dialium*) or the adaxial side (*Distemonanthus*, *Dicorynia*) of the flower. *Labichea punctata* retains only one lateral antesepalous stamen; examples are rare in which bilateral symmetry is not maintained even after loss of parts. A remarkable example of loss and reorganization of the remaining organs is in *Apuleia praecox*, which Thompson showed as regularly trimerous. Examples of radial trimery are known among mimosoids (Eichler, 1878) and *Gleditsia* (Tucker, unpublished).

Morphological loss of organs such as petals does not necessarily mean loss of genetic information for petal production. If one species has suppressed petals, other species of the genus often show variation in petal number (as in *Schotia*). Mutations often are known in which all petals enlarge. *Clitoria ternatea*, for example, in which the vexillum is usually the prominent petal, has a monogenic mutation in which all five petals become large and show the same color markings as the vexillum.

Suppression and loss of organs are quite different processes, although they are not always critically distinguished. Their respective effect on subsequent ontogenetic processes varies greatly in extent. At anthesis it is difficult to determine which process has been responsible for absence of organs. More examples need to be studied in early ontogeny, particularly among caesalpinioids, to analyze the consequences of loss versus suppression.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V. Conclusions

Developmental evidence is utilized to clarify the relationships among the subfamilies of legumes. Floral architecture differs: Mimosoideae show the median petal on the abaxial side, while the median petal in Papilionoideae and Caesalpinioideae is on the adaxial side. The order of organ initiation also differs. Sepals are initiated either simultaneously or helically among mimosoids, helical or rarely unidirectionally among caesalpinioids, and unidirectionally only among papilionoids. Petals and stamens are initiated in unidirectional order in each whorl in most caesalpinioids and all papilionoids. The moderate zygomorphy of caesalpinioid flowers can be correlated with the delay in appearance of zygomorphy until petal initiation; more marked zygomorphy characterizes papilionoids, in which zygomorphy is manifested at sepal initiation.

Precocity of specialized character states is itself considered a specialization (Gould, 1977). Papilionoideae and Caesalpinioideae are clearly close on the basis of developmental evidence. The caesalpinioids include both archaic features such as helical initiation and advanced ones such as unidirectionality. Papilionoids have specialized features, and these appear earlier in ontogeny than in caesalpinioids. Mimosoideae are isolated on developmental bases from the other two groups (particularly in the architecture of the flower), although they share with Caesalpinioideae the archaic feature of helical initiation of sepals in some taxa.

Tribal distinctions among Papilionoideae depend in part on whether stamens are free or fused. In all, stamens are free in early ontogeny; nine stamens may become fused (diadelphy) in some taxa, or all ten stamens may become fused (monadelphy or pseudomonadelphy) in other taxa. Pseudomonadelphy results from a diadelphous condition with additional surface fusion between the solitary free stamen and the adjacent sides of the stamen tube. Diadelphy is common in most tribes that show fusion, and most show either monadelphy or pseudomonadelphy as well. Of ten tribes including pseudomonadelphous taxa, all also have diadelphous taxa. Only three genera, in three different tribes, are reported to show all three conditions among their species: *Aeschynomene*, *Desmodium*, and *Erythrina*. These need to be studied and verified, as they seem to be exceptions to the generalization that pseudomonadelphy and monadelphy have arisen in separate lines. Among generic distinctions, the loss of certain organs (sepals, petals, or stamens) is an important one. In

← Figures 32–39. Floral organogenesis, organ loss and suppression in *Saraca* (SEM micrographs). Figures 32–36, 38, and 39 are *S. declinata;* Figure 37 is *S. indica*. Bracts and bracteoles are removed in all. Most or all sepals are removed in Figures 34–39. Abaxial side is at base in Figures 32–35, 37, and at right in Figures 36, 38, 39. —32. Five sepals have been initiated in helical succession (numbered, with "1" the oldest). Five primordia are initiating in petal positions (starred); bracteoles (B). —33. Five sepals are numbered in order of helical succession. First sepal (1) was removed as well as bracteoles (B). Five primordia (unlabeled) in petal positions surround the central carpel primordium (C); a primordium (starred) is in the lateral antesepalous stamen position. This is not usually the first member of this whorl to initiate. —34. Two adaxial sepals (2, 5) are fused by zonal growth and appear as one. Four stamen primordia (S) are in petal sites. Antesepalous primordia are initiating in abaxial and lateral sites (at arrows). —35. Flower with two whorls of organs around the central carpel (C). Sepals have been removed. The four larger primordia (S) are in petal sites but will become the functional stamens; three very small primordia (at arrows) alternating with the larger ones represent the outer stamen whorl, which will be suppressed. The arcuate ridge seen above the carpel is composed of three confluent primordia, two of the outer stamen whorl plus the standard petal rudiment. —36. Oblique view of slightly older flower showing four stamen primordia (S) in petal sites. The fifth petal primordium (P), in the site of the standard petal, is suppressed shortly after initiation. The arrow indicates one of the suppressed antesepalous primordia of the outer stamen whorl. —37. Flower of *Saraca indica* (at stage comparable to Fig. 35) showing a carpel (C) and seven primordia, all of which will become stamens: four in petal sites (P) and three in outer-stamen-whorl sites (S). —38, 39. Successively older stages of *S. declinata* flower enlargement. Carpel (C) is surrounded by a cup-shaped hypanthium with the four stamens attached to the rim. The antesepalous primordia are suppressed and appear only as slight thickenings (at arrows). Scale bars = 50 μm in Figures 32–37; = 500 μm in Figures 38, 39.

genera such as *Amorpha* and *Bauhinia*, the loss is only a matter of suppression. All organs are present, and they are initiated in the usual order and the normal sites, as in other related taxa.

The timing of suppression can vary; the earlier it occurs, the more profound the effect on subsequent development. Species of *Schotia* and *Saraca* show early suppression of a sepal and a petal, and at anthesis appear to be four-merous. In *Saraca* there are additional marked changes in the petal whorl, so that stamens are produced in petal sites. Transference of function also results after suppression in *Bauhinia divaricata*, in which nine stamen primordia are suppressed and later converted to form a staminodial tube around the carpel. In *Bauhinia malabarica*, selective suppression at mid-stage of development results in three floral morphs: male, female, and perfect.

In one legume studied, *Ateleia herbert-smithii*, there is complete loss of certain organs. Four of the five petals are lacking, and subsequent development is drastically disrupted in the sites of organs, in their order of initiation, and in number of organs. Loss of organs is far more significant developmentally than suppression of organs; loss of organs may have served as a mechanism of macroevolution in legumes.

Ontogenetic comparisons have been shown to provide insights on differences in floral structure and systematic relationships. The developmental basis for the major morphological distinctions among taxa (such as symmetry, position of organs, and their order of initiation) can be determined. The developmental differences, as well as their timing in development, can reveal how flowers of related taxa diverge among themselves. Innovations as well as heterochronic differences can be distinguished and their developmental bases analyzed. Such data presented in this paper provide useful insights for the pollination biologist who may be concerned with questions of convergent evolution and biology of legume flowers (see Schrire, this volume). Also stressed is the importance of cooperation among plant morphologists, systematists, and biologists to understand the phylogeny of the Leguminosae and their associated biota.

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References

Codd, L.E. 1956. The *Schotia* species of southern Africa. Bothalia 6: 515–532.

Derstine, K. S. & J. Ramírez-Domench. 1985. Significance of sepal and petal sites of mimosoid legumes. Amer. J. Bot. 72: 812.

——— & S. C. Tucker. 1985. Histological floral ontogeny of *Acacia baileyana* (Leguminosae: Acaciae). Amer. J. Bot. 72: 812–813.

Eichler, A.W. 1878. Blüthendiagramme. W. Engelmann, Leipzig.

Geesink, R. 1984. Scala Millettiearum. E. J. Brill/Leiden Univ. Press.

Gemmeke, V. 1982. Entwicklungsgeschichtliche Untersuchungen an Mimosaceen-Blüten. Bot. Jahrb. Syst. 103: 185–210.

Gillett, J. B. 1961. Notes on *Millettia* W. and A. in East Africa. Kew Bull. 15: 19–40.

Gould, S. J. 1977. Ontogeny and Phylogeny. Belknap Press, Harvard University, Boston.

Hanson, C. H. & W. A. Cope. 1955. Reproduction in the cleistogamous flowers of ten perennial species of *Lespedeza*. Amer. J. Bot. 42: 624–627.

Hilu, K. W. 1983. The role of single-gene mutations in the evolution of flowering plants. Evol. Biol. 16: 97–128.

HUTCHINSON, J. 1964. The Genera of Flowering Plants (Angiospermae). Volume 1. Dicotyledons. Oxford Univ. Press, Oxford, England.

JANZEN, D. H. 1981. The defenses of legumes against herbivores. Pp. 951–977 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Volume 2. Royal Botanic Gardens, Kew.

LORD, E. M. 1981. Cleistogamy: a tool for the study of floral morphogenesis, function, and evolution. Bot. Rev. (Lancaster) 47: 421–449.

MAIR, O. 1977. Zur Entwicklungsgeschichte monosymmetrischer Dicotylen-Blüten. Diss. Bot. 38: 1–90.

PERCIVAL, M. 1974. Floral ecology of coastal scrub in southeast Jamaica. Biotropica 6: 104–129.

POLHILL, R. M. & P. H. RAVEN (editors). 1981. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

RAMÍREZ-DOMENECH, J. & S. C. TUCKER. 1988. Patterns of organ development in mimosoid legume flowers. *In:* P. Leins, S. C. Tucker & P. Endress (editors), Aspects of Floral Development. Gebrüder Borntraeger, Stuttgart.

_____ & _____. Phylogenetic implications of inflorescence and floral ontogeny of *Mimosa strigillosa*. Amer. J. Bot. (submitted).

RUDD, V. E. 1968. A résumé of *Ateleia* and *Cyathostegia* (Leguminosae). Contr. U.S. Natl. Herb. 32: 385–411.

SOUSA S., M. & M. PENA DE SOUSA. 1981. New World Lonchocarpinae. Pp. 261–281 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Volume 2. Royal Botanic Gardens, Kew.

STEBBINS, G. L. 1974. Flowering Plants: Evolution Above the Species Level. Belknap Press, Harvard University, Cambridge.

TAUBERT, P. 1894. Leguminosae. *In* A. Engler & K. Prantl (editors), Die Natürlichen Pflanzenfamilien. Teil III, Abt. III. W. Engelmann, Leipzig.

THOMPSON, J.M. 1924. Studies in Advancing Sterility. 1. Amherstieae. Liverpool Univ., Hartley Botanical Laboratories Publ. 1: 1–54.

_____. 1925. Studies in Advancing Sterility. II. Cassieae. Liverpool Univ., Hartley Botanical Laboratories Publ. 2: 5–44.

TUCKER, S.C. 1984a. Origin of symmetry in flowers. Pp. 351–394 *in* R. A. White & W. C. Dickison (editors), Contemporary Problems in Plant Anatomy. Academic Press, New York.

_____. 1984b. Unidirectional organ initiation in leguminous flowers. Amer. J. Bot. 71: 1139–1148.

_____. 1987a. Pseudoracemes in papilionoid legumes: their nature, development, and variation. Bot. J. Linn. Soc. 95: 181–206.

_____. 1987b. Floral initiation and development in legumes. Pp. 183–239 *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

_____. 1988a. Heteromorphic flower development in *Neptunia pubescens*, a mimosoid legume. Amer. J. Bot. 75: 205–224.

_____. 1988b. Loss versus suppression of floral organs. *In* P. Leins, S. C. Tucker & P. Endress (editors), Aspects of Floral Development. Gebrüder Borntraeger, Stuttgart.

_____. 1988c. Dioecy in *Bauhinia* resulting from organ suppression. Amer. J. Bot. 75: 205–224.

_____, O. L. STEIN & K. S. DERSTINE. 1985. Floral development in *Caesalpinia* (Leguminosae). Amer. J. Bot. 72: 1424–1434.

UPHOF, J. C. T. 1938. Cleistogamic flowers. Bot. Rev. (Lancaster) 4: 21–49.

URBAN, I. 1885. Morphologie der Gattung *Bauhinia*. Ber. Deutsch. Bot. Ges. 3: 81–101.

WIT, H. C. D. DE. 1956. A revision of Malaysian Bauhinieae. Reinwardtia 3: 381–539.

ZUIJDERHOUDT, G. F. P. 1968. A revision of the genus *Saraca* (Legum.-Caes.). Blumea 15: 413–425.

STRUCTURE, EVOLUTION, AND BIOLOGY OF POLLEN IN LEGUMINOSAE

Ph. Guinet[1] and I. K. Ferguson[2]

Abstract

The pollen morphology of the Leguminosae shows that there are widely divergent morphologies, particularly at the generic level, in all of the three subfamilies. Pollen morphology is reviewed, giving more emphasis to common characters and trends and placing less emphasis on the nature of the dispersal unit (single versus compound grains). Four main trends are recognized: 1) changes in infratectal exine structure and apertural types; 2) increase in endexine thickness correlated with a decrease in or absence of the foot layer; 3) decrease of endexine thickness and a reduction of the aperture; 4) increase in the number of apertures. The fossil record, including much new data, is also reviewed. There is broad agreement between the changes in the fossil record, the variation in the pollen morphology of extant species, and the general diversity of the other characters in the family. Congruity between exine structure and the main apertural characters arose early in the fossil record and is indicative of a common ancestry in the family. In contrast, pollen exine sculpture (ornamentation of the wall) is less important in phylogenetic terms. The adaptive significance of the main pollen characters that are linked cannot be explained by phenotypic plasticity, and this is demonstrated by numerous examples. The role of external influences (e.g., pollination syndromes, ecological and abiotic factors on pollen change) appear more important than is currently recognized.

I. Introduction

The general trends and patterns of morphological changes and evolution in Leguminosae are fairly well known today as data accumulates from many well studied groups in the three subfamilies. Pollen structure and morphology of both living and fossil taxa have significantly contributed to understanding the systematics and evolution of this very large family, often reaffirming either the main subdivisions established by Bentham (1865) or the sequences he adopted. Furthermore, pollen morphology has permitted a correct placement of many of the numerous legume genera described after Bentham (Polhill & Raven, 1981).

Surveys of cryptic features, especially pollen, have provided a firm basis for understanding evolutionary patterns, and systems utilizing these characters frequently reflect natural affinities. Studies of pollen morphology have therefore contributed information that is valuable in practical applications (for example genetic diversity for plant breeding); however, they have also revealed a remarkable and unexpected diversity of both structural and apertural characters. This pollen diversity may be considerable even in some large genera known for the homogeneity of macromorphological characters, suggesting that the differences in pollen morphology have arisen by adaptive processes. A less tenable argument might be that as pollen function is relatively straightforward, such pollen diversity is the result of random variation and has become fixed by reasons of chance. It is the purpose of this review to try and summarize the recent pollen morphological studies in the Leguminosae, particularly as they relate to evolutionary biology.

[1]Laboratoire de Palynologie E.P.H.E., Université des Sciences et Techniques du Languedoc, 34060 Montpellier, Cédex, France.

[2]Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, England.

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II. Pollen Diversity

As in many angiosperm families in which pollination modes are predominantly biotic, the pollen characters in Leguminosae are extremely diverse. The array of this diversity is, on the whole, of the same amplitude as it is in several advanced orders of the Rosidae (e.g., Saxifragales) or of the Asteridae (especially Gentianales). Much of the pollen variation in the Leguminosae is due to the development of specialized pollen types, particularly in the subfamily Mimosoideae (variation in compound grains). Excluding these compound grains, the basic pollen type (Guinet, 1981a) common to the three subfamilies of the Leguminosae is single, isopolar, ± spheroidal, small sized (20–35 μm on longer axis) tricolporate grains with isodiametric endoapertures, with typical angiosperm exine stratification, a semitectate exine, a columellar infratectal structure, a well-developed foot layer with a thickness ± equal to the thickness of underlying endexine.

The fine structure of the apertural system provides a number of very useful indications of interrelationships between the subfamilies. For example, the inner exine beneath the apertures is modified in a characteristic manner, forming a "negative annulus" around the thickened internal pore, due to a thickened and recurved endexine. This can be seen by comparing Guinet (1978, fig. 6) with Patel et al. (1985, fig. 18). Although in the subfamily Mimosoideae the apertural thinning is generally lamellated with thin concentric strips at the pores, the overall modifications on the aperture are not restricted to this subfamily as previously thought (Guinet, 1969) but are common to the less specialized members of the three subfamilies, more especially in the Caesalpinioideae–Dimorphandreae (Ferguson, 1987), but also in *Jacqueshuberia* (Patel et al., 1985), *Vouacapoua* (Ferguson, 1987), and *Aldina* in the Papilionoideae (Ferguson & Skvarla, pers. comm.), as well as in many tropical Mimosoideae. Such local modifications, which are perhaps functionally comparable to those described in the apertural areas of the Sapindales (Melville, 1965) and Celastrales (Lobreau-Callen & Lugardon, 1983), occur independently of other modifications of the exine structure. They are absent in most temperate groups (predominantly the more advanced temperate Papilionoideae), however, and we have observed their absence in many cases, which we interpret as a loss, to be linked to a significant reduction of the endexine, at least at the apertures. However, this is not always the case, and it might be postulated that lower temperatures during pollen development (Rowley, 1976) may be an influence on morphology.

In spite of considerable variation in pollen morphology within the family, the main differences may be summarized as in Table 1.

III. General Trends of Pollen Changes

Although these trends have been based mainly on comparative pollen morphology of the extant Leguminosae, they agree with the broad trends in the family established from macromorphology, particularly with the specialization in both floral features (as illustrated by Polhill et al., 1981) and inflorescence structure. They are also in general agreement at a higher systematic level, with the main sequences of change of pollen characters as they appear in the fossil record (Table 2, Fig. 1), even though this record is obviously incomplete. In particular, the relative abundance of the fossil taxa referred to the Mimosoideae is an artifact due to unequal differentiation of the pollen characters in the three subfamilies. The pollen of Caesalpinioideae and Papilionoideae is often very difficult to identify at the generic level. The genera *Gleditsia, Gymnocladus, Zenia, Cercis, Dimorphandra,* and *Acrocarpus*—generally regarded as the most archaic in the family—are undetected in the fossil pollen record because their present-day species have a very generalized type, being tricolporate with a circular endoaperture, exine with a columellar structure, and they are shed in single grains (Graham & Barker, 1981; Larsen & Larsen, 1984; Ferguson, 1987).

TABLE 1. Summary of the main differences in pollen morphology between the three subfamilies.

	Caesalpinioideae	Mimosoideae	Papilionoideae
Infratectal exine structure	Usually columellar, occasionally granules and columellae, rarely granular *(Bauhinia)*	Usually granular	Usually columellar, occasionally granular (main exceptions in tropical groups)
Aperture configuration	Usually colpate or colporate, rarely pluriporate	Pluriporate, tricolporate in basic types (in both single and compound grains)	Usually colporate (three or more apertures), very rarely pluriporate
Aperture membrane	Not obviously thickened (no pseudoperculum or operculum). Exceptions: *Colophospermum*, *Hardwickia*, and *Sindora*	Not obviously thickened (no pseudoperculum or operculum). Exceptions: *Leucaena*, *Desmanthus*, and *Prosopis* groups (single grains with equatorial apertural thickening)	Frequently thickened (coarser granules, pseudoperculum or operculum)
Simple/compound pollen grains	Simple. Exceptions: *Afzelia* and *Diptychandra*	Usually compound (tetrads/polyads), simple in basic types	Simple
Ornamentation	Frequently striate, spines and spinules sometimes present	Usually areolate, occasionally striate (and associated with colpory). Spines and spinules absent	Usually reticulate, striae absent (a slight tendency towards striae in some *Swartzia*). Spines and spinules very infrequent

From the basic legume pollen type, pollen morphological series can be traced from analysis of the changes in the main characters.

EXINE STRUCTURE

Guinet (1981a) has suggested that the structure of the exine provides the most phylogenetically useful pollen character in the Leguminosae, a view that is upheld here because exine characters are very frequently associated with apertural characters (Guinet & Lugardon, 1976; Stainier & Horvat, 1978; Ferguson & Skvarla, 1983). It is proposed that in many cases structural changes in any layer of the exine seem to give rise to corresponding apertural changes. The reverse argument is hardly conceivable, as ontogenetic studies have clearly shown that apertures are just local modifications of the exine.

In Leguminosae, the exine almost always has a tectum that is usually continuous and reticulate. The semitectate type (Fig. 2) is regarded as basic because of both its occurrence and position in the fossil record and its presence in the extant most archaic genera.

The type of tectum is very often related to the exine structure. For example, semitectate types are most frequently associated with a columellar structure, while the other sculptural types are generally not. This is particularly the case for the large array of ornamentation types present on the tectum (supratectal reticulum, areolae, verrucae, for example) where the infratectal structure is granular.

An intectate exine sensu stricto occurs in the single monotypic genus *Dinizia* (Mimosoideae)

TABLE 2. Selected pollen characters from the fossil pollen record in the Leguminosae. The fossil record is taken from Muller (1981) and supplemented (see notes) by more recent findings. All the references included in Muller (1981) have been omitted. The first column refers to fossil taxa, while the second refers to taxonomic groups recognized by Polhill & Raven (1981). The pollen characters of the fossil taxa are those described or illustrated in original publications. In column 1, boldface characters = Caesalpinioideae, italics = Mimosoideae; Papilionoideae are represented by the last two taxa in columns 1 and 2. In column 4, EUR = Europe, AM = America, AS = Asia, AFR = Africa, AUST = Australia. In column 5, COL = columellate, GR = granular.

Fossil types	Affinities (present groups)	Oldest occurrence		Exine	Apertures	Pollen unit	Ornamentation
Sindora	Detarieae, Detarium group (Sindora)	Maestrichtian[1]	EUR-AM	COL	3 colpi, 6 circular pores	single grain	reticulate
Crudia	Detarieae, Crudia group	Paleocene	AFR-AM	COL	3 colporate, circular pores	single grain	striate
Maniltoa grandiflora	Detarieae, Cynometra group	Paleocene	AM	COL	3 colporate	single grain	reticulate
Protomimosoidea buchananensis	Mimoseae[2]	Paleocene-Eocene boundary	AM	COL	3 colporate	single grain	smooth (perforate)
Caesalpinia	Caesalpinia group	Lower Eocene	AM-AS	COL	3 colporate, elliptic pores	single grain	reticulate
Pentaclethra macrophylla	Parkieae (Pentaclethra)	Lower-Middle Eocene	AFR	COL	3 porate[3]	single grain	subareolate
Brachystegia	Amherstieae, Brachystegia group	Middle Eocene	AFR	COL	3 colporate, circular pores	single grain	broad reticulate
Eomimosoidea	Unknown[5]	Middle Eocene	AM	COL	3 colporate	tetrad	areolate
Amblygonocarpus	Adenanthera group	Middle Eocene	AFR	GR	distal pores	16-grain pollen	areolate
Cassia[6]	Cassieae (Cassia s.l.)	Middle Eocene	EUR	COL	3 colporate	single grain	subsmooth
Tetrapleura	Adenanthera group (Tetrapleura)	Middle Eocene	AFR	GR	distal pores	16-grain pollen	smooth
Neptunia oleracea[6]	Dichrostachys group (Neptunia oleracea)	Middle Eocene	EUR	COL	pluricolporate	single grain	substriate

Unnamed[7]	affinities unknown	Middle Eocene	EUR	COL	3 colporate, circular pores	single grain	reticulate
Albizia[4]	Ingeae, Albizia group	Middle-Upper Eocene	AFR-AM	GR	pluriporate	16-grain pollen	areolate
Parkia	Parkieae (Parkia sect. Sphaeroparkia)	Upper Eocene	AFR	COL	distal pores	16-grain pollen	interrupted reticulate
Calpocalyx ngouniensis	Xylia group (Calpocalyx)	Upper Eocene	AFR	COL	distal colporate	8-grain pollen	areolate-verrucate
Pseudoprosopis sericeus	Adenanthera group	Upper Eocene	AFR	GR	distal porate	8-grain pollen	areolate
Adenanthera	Adenanthera group	Upper Eocene	AFR	GR	distal porate	12, 8-grain pollen	smooth
Xylia[4]	Xylia group (Xylia)	Upper Eocene	AFR	GR	distal porate	8-grain pollen	rugulate
Acacia, type I	Acacieae, Acacia subg. Aculeiferum	Upper Eocene	AFR	GR	pluriporate	16-grain pollen	smooth
Fillaeopsis	Mimosae (Fillaeopsis)	Oligocene	AFR	COL	3 colporate	tetrad	reticulate
Calpocalyx brevibracteatus[4]	Xylia group (Calpocalyx)	Oligocene	AFR	COL	distal porate	8-grain pollen	smooth
Leucaena	Leucaena group (Leucaena ?)	Oligocene	AFR	COL	3 colporate	single grain	smooth
Acacia, type III	Acacieae, Acacia subg. Acacia	Oligocene	AM	COL	pluriporate	16-grain pollen	smooth (perforate)
Prosopis juliflora	Mimoseae, Prosopis group (Prosopis)	Lower Oligocene	AM	COL	3 colporate	single grain	smooth (perforate)
Prosopis farcta[8]	Mimoseae, Prosopis group (subg. Prosopis)	Oligo-Miocene	EUR	COL	3 colporate	single grain	areolate

TABLE 2. Continued.

Fossil types	Affinities (present groups)	Oldest occurrence		Exine	Apertures	Pollen unit	Ornamentation
Ceratonia[9]	Cassicae, Ceratonia	Oligo-Miocene	EUR	COL	4 colporate	single grain	reticulate
Eperua	Detarieae (Eperua)	Lower Miocene	AM	COL	3 colporate	single grain	reticulate
Acacia, type II	Acacieae, Acacia, subg. Phyllodineae	Lower Miocene	AUST	GR	pluriporate + pseudocolpi	16-, 8-grain pollen	reticulate
Desmanthus	Dichrostachys group (Desmanthus)	Upper Miocene	AM	COL	3 colporate	single grain	striate
Archidendron	Ingeae (Archidendron and allied genera)[10]	Pliocene	AS	GR	pluriporate	16-grain pollen	areolate
Calliandra[11]	Ingeae (Calliandra)	Pliocene	AM	?	distal porate	8-grain pollen	subsmooth
Indigofera	Indigofereae	Pliocene	AFR	?	3 colporate	single grain	?
Astragalus	Galegeae	Pliocene	AFR	?	3 colporate	single grain	?

[1]Or Santonian–Coniacian (see Muller, 1981).

[2]Crepet & Taylor (1985, 1986).

[3]The pollen of this species has been described as having "indistinct short colpi (Muller, 1981). The "colpi" are actually so short (less than the diameter of the underlying pores), that we prefer to include this type in the porate category.

[4]Guinet et al., in press.

[5]Daghlian et al., 1980. This fossil genus has many pollen characters in common with the monospecific extant African genus *Fillaeopsis*. The only distinguishing pollen character is the verrucate tectal surface (reticulate in *Fillaeopsis*).

[6]Guinet & Gruas-Cavagnetto, 1986.

[7]Gruas-Cavagnetto, unpublished.

[8]Guinet & Bessedik, 1984.

[9]Bessedik, 1985.

[10]Nielson et al., 1984b.

[11]Hooghiemstra, 1984. Oldest known pollen record: 3,370,000 yrs. B.P. Pollen analysis of the sediments of the lower part of the basin of Bogota may extend this record downwards (Hooghiemstra, pers. comm.).

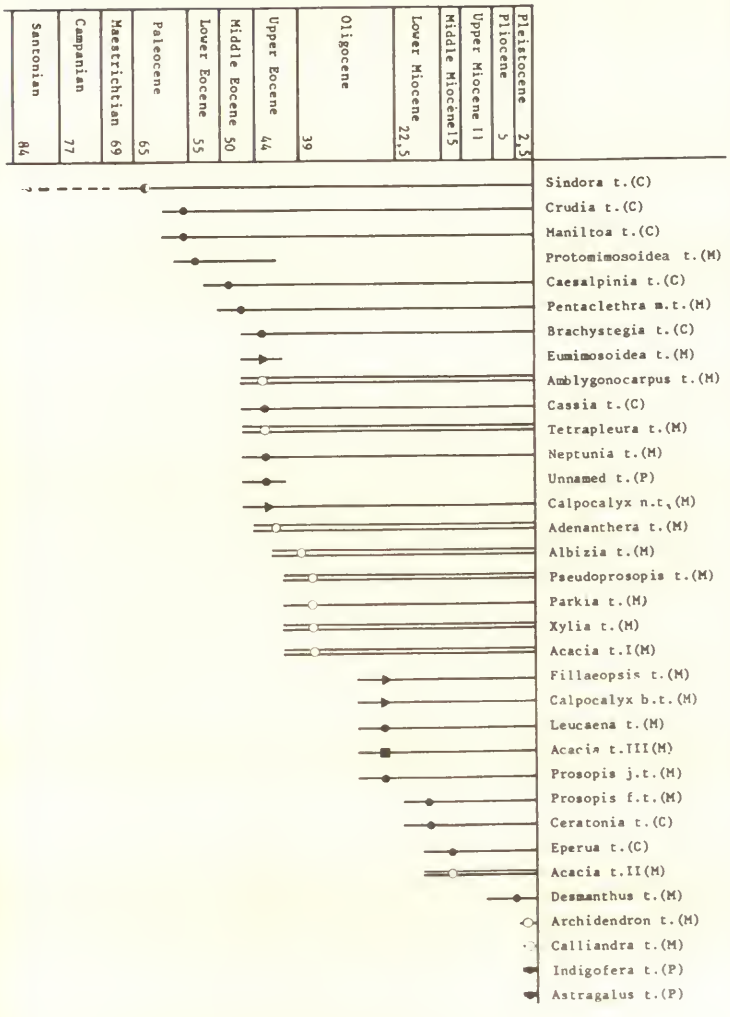

FIGURE 1. Changes in the pollen characters, as seen from the fossil pollen record. Modified from Muller (1981). Solid line = columellar infratectal exine structure, double line = granular infratectal exine structure, broken line = infratectal exine structure unknown. Half-shaded circle = the *Sindora* type (see Table 2), single grains; shaded circle = three equatorial colpori, single grains; triangle = three equatorial colpori, compound grains; square = three nonequatorial colpori, compound grains; open circle = three nonequatorial pores, compound grains. The pollen characters shown here have been observed or described from the fossil pollen record. Due to the very great variation in pollen characters (both exine structure and apertural type), they rarely reflect the pollen characters at the generic level. This diagram shows that the tricolporate single grain is basic. The most important pollen changes (exine structure, apertural type, single versus compound grains) occur during the Upper Eocene. The Oligocene (drier climatic conditions) is marked by a greater radiation of the taxa with compound grains. C = Caesalpinioideae, M = Mimosoideae, P = Papilionoideae.

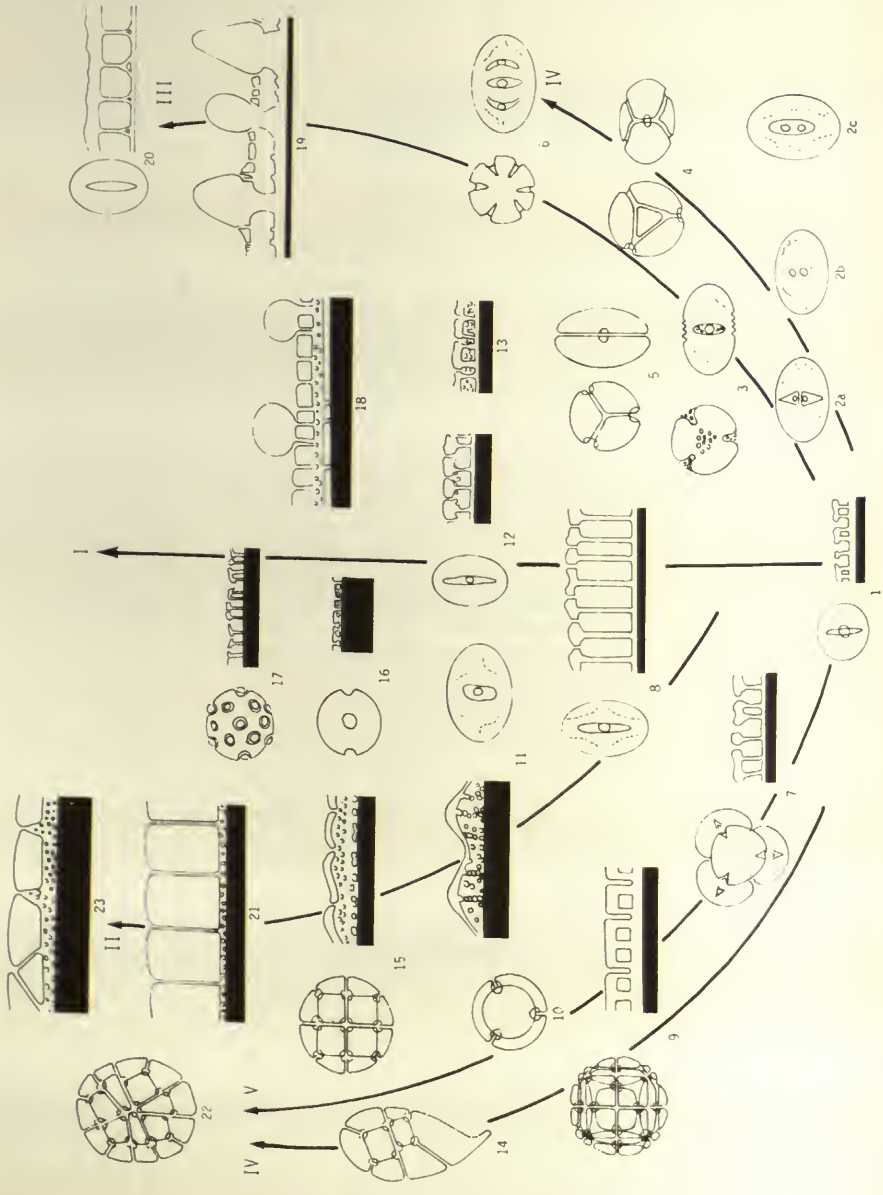

FIGURE 2. Diagrammatic representation of the main pollen characters and their evolutionary changes in the Leguminosae. (Pollen size not to scale. Exine structure: ectexine in white, endexine in black). Main trends: **I.** Change in infratectal exine structure from columellar to granular. The change is generally linked with a decrease or absence of the foot layer and a change in the apertural type from colporate to porate (1, 11, 12, 13, 15, 21, 23). **II.** Increase in the endexine thickness, correlated with a reduction or absence of the foot layer (1, 11, 15, 16, 17, 21, 23). **III.** Decrease in the endexine thickness and simplification of the apertural system (19, 20). **IV.** Increase in the aperture number by duplication of the endoapertures in each colpus (2) or by equatorial increase of the number of colpori (6). At left: nonequatorial increase of the aperture number in compound grains (9, 14, 15, 22). **V.** Change from single to compound grains (1, 7, 9, 10, 14, 15, 22). In derived groups, this change is associated with pluripory. —1. The basic type: tricolporate single grain with a circular endoaperture. A "complete" semitectate exine with a columellar structure. Foot layer of the same thickness as the endexine. The common type in the three subfamilies. —2. Duplication of the endoaperture number within each of the three equatorial colpi. A similar exine structure. 2a, from *Sindora* (Caesalpinioideae); 2b, from *Dumasia* (Papilionoideae); 2c, from *Aeschynomene* (Papilionoideae). —3. Pseudosyncolpy. The colpi ends are distinct. The polar modifications of the tectum are ± similar to ornamentation of the colpi membranes. From *Canavalia* (Papilionoideae). —4. Parasyncolpy. In the Leguminosae this character is linked with breviaxy and a columellar exine structure. —5. Syncolpy. Again linked with a columellar exine structure. Syncolpy occurs in the tricolporate type, but also in the tricolpate type. From *Arachis* (Papilionoideae). —6. Equatorial increase of compound apertures. In many Papilionoideae with a columellar exine structure. From *Clitoria* (Papilionoideae). —7. Tricolporate stable tetrad. Three equatorial colpori on each grain. A columellar exine structure. From *Fillaeopsis* (Mimosoideae). —8. Columellar infratectal exine structure with well developed and fine columellae. From *Leucaena*, single grain group (Mimosoideae). —9. Tectate perforate exine with a columellar structure. Sixteen colporate (syncopate) grain polyad. From *Acacia* subg. *Acacia* (Mimosoideae). —10. Tetrad with three equatorial pores on each grain. Exine infratectal structure: mixed or granular. From *Mimosa* (Mimosoideae). —11. Change in exine infratectal structure; granules partly arranged in radially oriented rows. Foot layer absent. From *Vigna* (Papilionoideae). —12. Change in exine infratectal structure; columellae and granules mixed. Granules more abundant near the tectum and merging with it. From *Swartzia* (Papilionoideae). —13. Change in exine infratectal structure; intercolumellar cavities filled with granules. From *Calpogonium* (Papilionoideae). —14. Large 8-grain polyad with a grain modified with an appendix (a "tail cell"). Exine infratectal structure: mixed (13) or columellar. From *Calliandra* (Mimosoideae). —15. A 16-grain polyad with a discontinuous foot layer (broad granules). Nonequatorial and nonequidistant pores. From *Acacia*, subg. *Aculeiferum* (Mimosoideae). —16. Very reduced ectexine. A columellar structure (short columellae) associated with a very thick endexine. "Fading" of the colpi. From *Erythrina* (Papilionoideae). —17. An unusual type in the Leguminosae. Pluriporate grain, with a columellar infratectal exine structure and very fine columellae, foot layer absent and a very thick endexine. From *Cranocarpus* (Papilionoideae). —18. Supratectal verrucae on a perforate tectum. A granular infratectal structure, ± porate apertures (indistinct colpi). From *Bauhinia* (Caesalpinioideae). —19. "Columellar" broad verrucae and spines. A well developed foot layer. Apertures: as in 6. From *Bauhinia* (Caesalpinioideae). —20. The colpate type: three equatorial colpi. No endexine. From *Geissapsis* (Papilionoideae). —21. Very thick perforated tectum: isodiametric, radially oriented channels. Foot layer absent, or nearly so. Apertures: pores. From *Pithecellobium* (Mimosoideae). —22. A 28-grain polyad. Not infrequent in the Mimosoideae–Ingeae. Exine infratectal structure as in 21 or 23. —23. Nonisodiametric and nonradially oriented tectal channels. A granular infratectum. Pluriporate apertures. From *Archidendron* (Mimosoideae).

(Guinet, in prep.). The tectum is generally patterned (although smooth in species or genera scattered throughout the three subfamilies) and sometimes has complex structure. For example in *Swartzia* (Ferguson, 1984) and perhaps in *Baphia* (Soladoye & Crane, 1985), both primitive Papilionoideae, the tectum appears more or less spongy or "granular," with an underlying irregular columellar layer. In many groups the tectum is entirely crossed by a variable number of channels (pits), either randomly scattered, in smooth or weakly sculptured exines, or showing a distinct pattern delimiting the raised (thicker) tectal areas. This occurs in many Mimosoideae with an areolate tectum, in *Eperua* in the Caesalpinioideae (Cowan, 1975), and in *Castanospermum, Desmodium,* and *Indigofera* in the Papilionoideae (Ferguson & Skvarla, 1981; Ferguson & Strachan, 1982). These communication channels are particularly frequent and numerous in thick exines (tectum 5–8 μm). In the Mimosoideae–Ingeae, they are sometimes obviously broader in their proximal parts and are nonradially orientated in *Archidendron* and allied genera (Nielsen et al., 1983a, 1984b; Niezgoda et al., 1983).

Two main types of structure may occur under the tectum. These are the columellar and the granular types. A typical angiosperm columellar infratectal structure comprised of isometric, rodlike, well separated elements, generally higher than broad, occurs in the three subfamilies. However, it is much more frequent in the Caesalpinioideae and Papilionoideae, while a typical granular infratectal structure is most common in the Mimosoideae. However, it must be emphasized that as TEM data accumulate, more and more variations are found in these two infratectal types (Guinet & Lugardon, 1976; Ferguson & Skvarla, 1983; Niezgoda et al., 1983; Caccavari, 1986; Ferguson, 1987). Either the "columellae" lack their typical rodlike shape (e.g., *Acacia, Calliandra, Vicia, Lotus, Clitoria*) or the granules are radially ordered and fused (*Vigna,* and some other Papilionoideae), sometimes forming a "spongy" layer (Stainier & Horvat, 1983). Not infrequently, a mixture of columellae and granules occurs where granules fill the intercolumellar cavities. Such "mixed" structures have been noted in *Gymnocladus, Campsiandra, Erythrophleum, Stemonocleus, Pentaclethra, Mimozyganthus, Swartzia* (Ferguson & Guinet, unpublished), as well as in *Vigna* (Horvat & Stainier, 1979) and in *Calliandra* (Niezgoda et al., 1983). Their occurrence may indicate intergeneric or intertribal transitions.

A foot layer is generally present in the Leguminosae, it is usually homogeneous, apart from the presence of internal foramina (as defined by Skvarla & Turner, 1966), which occur in all three subfamilies. The foramina do not seem to have taxonomic significance but are useful, even in light microscopy, to draw the limits between foot layer and endexine proper. Very rarely (*Jacqueshuberia* [Caesalpinioideae], Patel et al., 1985; *Bauhinia* s.l. [Caesalpinioideae], Ferguson & Pearce, 1986) the foot layer is crossed by numerous isometric channels. In *Diptychandra* (Caesalpinioideae) there are well-developed communication channels (Guinet, 1965; Ferguson, 1987) connecting the individual pollen grains in the tetrad; these pass through both the foot layer and a well-developed endexine layer. In a few taxa the foot layer is discontinuous or coarsely lamellate (*Vicia, Desmodium, Euchresta* [Papilionoideae]—Ferguson & Skvarla, 1981; Ferguson, 1986) and is frequently reduced in thickness when the endexine is well developed.

The endexine does not show structural variations, except in apertural areas, and it usually only varies in thickness or in presence or absence. The only exception is in the genus *Jacqueshuberia,* where the endexine is clearly lamellated (Patel et al., 1985). The structural stability of the endexine may perhaps explain why the characters linked to it must be viewed as more "conservative."

Apertures

One of the most interesting features of the apertures is their fine structure, which appears to place the Leguminosae more closely with the Sapindales–Saxifragales than with the Rosales (Dickison, 1981; Polhill et al., 1981). However, the absence of TEM data in the Sapindales precludes any further comparison. Since the characteristic features of the fine structure of the apertures have been

retained better in the tropical representatives of the family, they provide a link between the most ancient *tropical* members of the three subfamilies.

Equidistant apertures

The most widespread and basic type of aperture in the fossil record and among extant species is colporate with equidistant, equatorial, circular endoapertures in the center of each colpus. The fossil record suggests that the nonisodiametric endoaperture as well as the H-shaped endoaperture found throughout *Indigofera* (Ferguson & Strachan, 1982) are derived. The porate type, with three equidistant equatorial pori, is less frequent but occurs in the Mimosoideae, where it is associated with compound units comprised of a low number of grains (tetrads, 8- or 12-grain polyads). These predominate in the tribe Mimoseae. In the Mimosoideae, porate apertures are very rarely associated with single pollen grains. Porate pollen is infrequent in the Caesalpinioideae but present especially in the genus *Bauhinia*. In the Papilionoideae it occurs primarily in the more derived tribes Phaseoleae and Desmodieae.

An equatorial increase of aperture number without change in exine structure is a characteristic feature of the Papilionoideae, and up to seven can occur. Nonequatorial increase of the aperture number may arise in the Leguminosae in several ways, all of which are infrequent. (1) The overall increase of the aperture number with shorter ectoapertures is generally associated with a change in the shape of the grains in Trigonellae (Papilionoideae) (Small et al., 1981). The pericolporate types (which occur together with the tricolporate ones in a single stamen, i.e., in *Trigonella*), appear only as a variant. (2) By fusion and subdivision of the colpi (ectoapertures) in the genus *Ceratonia* (Caesalpinioideae), the pollen may have 12 fused colpi, usually four with typical endoapertures (Graham et al., 1980; Ferguson, 1980a), and also by increase of aperture number in the same species, as in *Neptunia oleracea* in Mimosoideae (Guinet, 1969). (3) By increase of the endoaperture number inside each colpus (circular pores arranged in two parallel rows away from the equator). This last type occurs in all three subfamilies: *Sindora* in Caesalpinioideae (Van Campo, 1963); *Leucaena* and *Prosopis* in Mimosoideae (Guinet, 1969); and in *Dumasia* (Ikuse, 1956) and *Aeschynomene* in Papilionoideae (Ferguson & Skvarla, 1981; Guinet & Ferguson, unpublished). Periporate pollen is rare in the Leguminosae. In the Caesalpinioideae it occurs in *Hardwickia, Bauhinia (Piliostigma),* and *Colophospermum* (Graham et al., 1980); and in the Papilionoideae in *Cranocarpus* (Ferguson, 1978; Ferguson & Skvarla, 1979).

Nonequidistant apertures

In many Mimosoideae, where the pollen is shed in compound units, there are often 4–12 apertures differentiated on each grain[3] that are not equally spaced. Some apertures are distally or subdistally located, i.e., near the external surface of the polyad; others are "internal," i.e., near or at the proximal pole of each grain. All are functional, as shown by Coetzee (1955) in the genus *Acacia*. These apertures may be all of the same type (e.g., distal and proximal circular pores in the greater part of the Ingeae) or of two types. In *Acacia* subg. *Acacia*, each grain is distally colporate but only porate on the proximal and lateral areas. The reason for this apertural heterogeneity appears to be due only to the differences in exine structure. We interpret these dissymmetric types with more than three apertures as the result of the physical constraints due to the compound nature of the pollen (Wodehouse, 1935) and as homologous to the periaperturate condition in single grains.

[3]We have avoided, for sake of clarity, the term monad, which has been used in two opposite ways (Erdtman, 1969; Van Campo & Guinet, 1961) and have adopted "grains" instead of "cells." A single unit of a compound grain is equivalent to a single grain.

Syncolpy, parasyncolpy

"Fusion" of the colpi at or near their ends is not infrequent in the Leguminosae. It generally occurs at both poles (isopolar single grains) and mainly in the subfamily Papilionoideae (widely in Tephrosieae, Dalbergieae, Swartzieae, and also in Phaseoleae and Aeschynomeneae). It occurs much less frequently in Caesalpinioideae in *Cassia, Eligmocarpus* (Senesse, 1980), and in *Baikiaea* (Graham & Barker, 1981) and Mimosoideae *(Neptunia, Desmanthus).* Syncolpy is largely correlated with the shape of the grains, typically occurring in prolate grains. In oblate and peroblate pollen ("breviaxes"), syncolpy is often replaced by parasyncolpy, as occurs frequently in the Sapindales and Myrtales. In polymorphic species such as *Neptunia oleracea* (Mimosoideae), prolate grains are generally syncolpate while the oblate ones are parasyncolpate (Guinet, unpublished).

In many oblate or peroblate grains in the Papilionoideae, "fusion" of the colpi is due to an independent modification of the exine (thinning of the tectum and its fragmentation), occurring either at the two polar areas or on a single pole, as demonstrated by Kavanagh & Ferguson (1981) in *Canavalia.* Such dissymmetric modifications are also frequent in the Myrtales (Patel et al., 1984) and Sapindales (Muller & Leenhouts, 1976). In the Papilionoideae the rounded ends of the short colpi are generally distinct and, consequently, we prefer to coin the term "pseudosyncolpy" to name the polar modifications that cannot be interpreted as the coalescence of the ends of the colpi. Pseudosyncolpy occurs in Papilionoideae in large grains with a thick exine. The presence of such dissymmetric patterns as syncolpy, parasyncolpy, and pseudosyncolpy on a single pole (heteropolarity) is difficult to explain. Although we cannot demonstrate this ontogenetically, we suggest, by comparison with the differential deposition of ectexine on each grain when the pollen is compound (e.g., *Acacia*), that the pole where colpi are reduced or absent is the proximal one; that is the pole furthest from the tapetal source of sporopollenin during pollen wall development. A similar situation occurs in Orchidaceae (Cocucci & Jensen, 1969), in which the inner tetrads of a single pollinium, which are most remote from the tapetum, lack ectexine.

Parasyncolpy is not necessarily related to syncolpy and frequently occurs in the Myrtales and the Sapindales (but not in the Rosales). It is generally linked to breviaxy in the Leguminosae (as well as in the Myrtales and Sapindales). In the Leguminosae it must represent a novel but uncommon mechanism for a nonequatorial increase of the aperture number from the colporate type. This mechanism is perhaps comparable to the successiform model described by Van Campo (1967, 1976), the only important difference being that in the Leguminosae "successiformy" derives from a colporate aperture rather than a simple colpus. In *Ceratonia siliqua* (Caesalpinioideae), a very polymorphic species (Ferguson, 1980a), 12-pericolporate grains may occur (each colpus representing a semicolpus), with usually only four having typical endoapertures. In *Neptunia oleracea* (Mimosoideae), similar variation is present (Guinet & Gruas-Cavagnetto, 1986).

Heterocolpy

Subsidiary colpi, i.e. colpi-like superficial modifications of the ectexine with no germinative function, occur in the three subfamilies. Pseudocolpi are infrequent in the Caesalpinioideae, occurring in *Afzelia* (Senesse, 1976), but are more frequent in the Mimosoideae (Mimoseae and Acacieae) and in the Papilionoideae (Phaseoleae and Trifolieae). When the pollen is shed in single grains, pseudocolpi are located at the equator (Caesalpinioideae and Papilionoideae; no species of the Mimosoideae with single grains have pseudocolpi). Here generally three pseudocolpi regularly alternate with three germinal apertures. In several species of the genera *Phaseolus* (Bronkers, 1967) and *Trifolium* (El Baba, 1982), there are six equatorial pseudocolpi, two between adjacent colpori. *Nesphostylis holosericea* has remarkable pollen in the Papilionoideae, with six equatorial pseudocolpi fused in pairs. In extraporate (pseudocolpi together with independent pores) compound pollen of the Mimosoideae, pseudocolpi occur on the distal surface of the polyads (at the distal poles

of each grain) where the ectexine is fully developed, present as a continuous layer. This occurs in some species of *Piptadenia, Pseudopiptadenia, Stryphnodendron,* and many *Acacia* species (Guinet, 1978, 1986). In the subgenus Phyllodineae of *Acacia,* more or less distinct extra-apertural depressions connect the distinctly extraporate type with the porate one. Here, species and species groups (Bentham's Series) with pollen devoid of pseudocolpi are predominant in areas with more equable, tropical, wetter climates (Guinet, 1986).

As judged from the distribution and occurrence of these aperture-dependent (syncolpy, parasyncolpy) or independent (pseudosyncolpy, heterocolpy) modifications of the exine, they appear as independently advanced states in the Leguminosae. In the Papilionoideae, they are absent in species and genera with short colpi as well as in the porate taxa. In the Mimosoideae, in which pseudosyncolpy is unknown, they occur in the porate compound types exclusively when the infratectal structure is granular. One may postulate that the harmomegathic function, when it cannot be accommodated by the colpi (under comparable exine structures), could be transferred to more flexible polar areas (Ferguson, 1980b) or else to a greater number of pseudocolpi. The increase in the surface area of or the number of pseudocolpi is then viewed as a response to an increase in the harmomegathic needs. This would suggest a distinct and more sensitive response of the thinnings located in the ectexine (colpi and/or pseudocolpi) to environmental influences.

EXINE ORNAMENTATION

Sculpturing of the exposed surface of the pollen wall (the distal walls of each grain when the pollen is compound) exhibits considerable variation in the Leguminosae. All the major types known in the angiosperms have been observed (psilate, scabrate, verrucate, gemmate, clavate, fossulate, rugulate, areolate, striate, and reticulate in which every type of transition may occur). The areolate[4] type is not restricted to the Leguminosae and frequently is referred to in the Leguminosae, as well as in many angiosperm families, as verrucate.

Such a great diversity cannot be explained only by the great size of the family. The Compositae and Orchidaceae have more genera and species, and their general pollen characters are comparatively more uniform, except for the patterns of the supratectal structures. The pollen (apertural types and exine structures) is relatively uniform in the Orchidaceae (Schill & Pfeiffer, 1977; Burns-Balogh, 1983; Burns-Balogh & Funk, 1986), a family well known for the occurrence of narrow and constant pollination syndromes. Here, as in most Leguminosae, exine sculpture is unrelated to changes in exine structure, but similarities in superficial (supratectal) sculptural types between the Orchidaceae and the Leguminosae strongly suggest the importance of external factors, particularly pollination modes, for the explanation of such a diverse variation in apparently very dissimilar sculptural types.

The greatest pollen diversity at the generic level, irrespective of the number of the species involved, is known in the subfamily Caesalpinioideae, mainly in the *Bauhinia* group (Schmitz, 1973; Larsen, 1975; Ferguson & Pearce, 1986), in which nearly all the sculptural types observed in the Leguminosae are present except the clavate type (nonsupratectal clavae), which occurs in the genus *Dinizia* (Mimosoideae) (Feuer, 1987). Supratectal clavae occur as a primary sculpture type in unrelated groups, as in *Bauhinia* s.l. (Caesalpinioideae) (Ferguson & Pearce, 1986) and in *Macrotyloma* and *Herpyza* (Papilionoideae) (Ferguson, 1981; Kavanagh & Ferguson, 1981). But here the distinction between clavae, gemmae, and broad verrucae is often a question of morphological limits. Small clavae, gemmae, and verrucae may occur as secondary sculpture in very coarsely reticulate exines, and many pollen with open reticulate ornamentation frequently have free exine elements in the lumina. These characters are associated with large pollen size and are present

[4]Areolate: with small sexinous, usually circular or polygonal areas separated by grooves ± forming a "negative reticulum." In Erdtman (1947), a term suggested by Iversen.

in several Caesalpinioideae (e.g., *Arapatiella, Arcoa, Afzelia, Baikiaea, Brachystegia, Chidlowia, Delonix, Eperua, Intsia* [Graham & Barker, 1981], and *Jacqueshuberia* [Patel et al., 1985]; in a few Mimosoideae (e.g., *Parkia, Dichrostachys* [Guinet, 1969], and *Gagnebina* [Lewis & Guinet, 1986]); and in Papilionoideae (e.g., in *Erythrina* [Hemsley & Ferguson, 1985], *Ramirezella* [Maréchal et al., 1978], and *Dunbaria* [Thuan, 1973]). Transitions between this type and the rugulate forms ("reticulum" with discontinuous muri) occur isolated in species or species groups in unrelated genera (e.g., *Erythrina, Dichrostachys*, and *Parkia*). Such characters, when they occur together, are much more frequent in the taxa pollinated by large pollinators (Graham & Barker, 1981; Hemsley & Ferguson, 1985; Hopkins, 1984; Patel et al., 1985). They appear at the generic and subgeneric level as the markers of a similar, convergent, selective response, under common constraints to a single external factor.

Striate exines are predominant in the Caesalpinioideae, their greater frequency is noteworthy in Amherstieae (the *Berlinia* group) and in Cercideae (*Bauhinia* s.l.). But faint striae recur in the Mimosoideae in three related genera *(Neptunia, Desmanthus, Entada)* that do not appear to be closely related to the Caesalpinioideae. Moreover, the change from a finely reticulate exine to a striate type is known in the genus *Entada*, and the change from striate to coarsely rugulate surfaces is known in *Neptunia oleracea*. The striate pattern is frequently associated, as in many other families of the Rosidae, with uniformly distributed exine thickness (Muller, 1979) and, in the Leguminosae, with a columellar structure. Striations, however, appear to be absent from the subfamily Papilionoideae, with the possible exception of some species of *Swartzia*.

The echinate type is infrequent in the Leguminosae. Very small supratectal spinules occur only in *Bauhinia* s.l. *(Piliostigma), Hardwickia*, and *Colophospermum*. Echinules (as well as large spines) are completely absent from the Mimosoideae. They are just "tectal outgrowths," unrelated to the exine structure of the underlying layers. In many wind-pollinated angiosperms they are associated with a smaller pollen size and frequently with porate apertures. It is therefore worth investigating Ross's (1977) suggestion that *Colophospermum* is wind-pollinated. *Harleyodendron* has pollen with "incipient" tectal spinules (Ferguson & Skvarla, 1981, plate 2, fig. C) due only to modification of the tectum. The pollination mechanism for this genus is unknown but likely to be entomophilous. *Macrotyloma* appears to be the only genus in the Papilionoideae with pollen covered by large supratectal spines (Ferguson, 1981), while in some species of *Bauhinia* there are large, apparently supratectal verrucae (Ferguson & Pearce, 1986). The large spines that occur in some other *Bauhinia* species are not tectal spines but very different "columellar spines" directly "arising" from the foot layer. These spines are thus directly related to the infratectal exine structure (Ferguson & Pearce, 1986). This would suggest a distinct ontogenetic development, which may follow the highly evolved scheme described by Rowley & Dahl (1977) in the genus *Artemisia* (Compositae), but apparently much more frequent in the advanced angiosperms in which biotic pollination predominates (Sterculiaceae, Malvaceae, Convolvulaceae, Dipsacaceae, Compositae). These apparently very different echinate types, whose development seems related to pollination ecology, are certainly congruent with other characters. In *Macrotyloma* (Ferguson, 1981) the change in the exine infratectal structure from columellar to granular is also combined with the change from colpory to pory. In this genus the large supratectal spines are associated with porate apertures, while colporate apertures have small spines. Very short spinules and very large spines must represent the extreme morphological expressions of opposite trends. Spines and spinules are always associated in the Leguminosae with another type of sculpture, most frequently a small reticulate pattern. This reticulate type sculpture is perhaps more significant in phylogenetic terms than the development of spines even when these are apparently the dominant sculpture.

Smooth or subsmooth exine surfaces are not infrequent in the three subfamilies, even in groups pollinated by bees or birds. They are generally associated in the Leguminosae with comparatively large-sized pollen grains and a well-developed, thick, perforated tectum (Mimosoideae: Ingeae; Papilionoideae: Diocleinae). Such characters frequently occur in taxa found within or at the margins of tropical open forests.

Areolate ornamentation that is sometimes hard to separate from the verrucate type (e.g., in the small-sized compound grains of the genus *Mimosa*) is frequent in the Leguminosae. It occurs in the three subfamilies but is much more common in the Mimosoideae and is particularly well developed in the Ingeae. In this tribe the areole shape is very variable, being circular, polygonal, to even an intricate "jigsaw puzzle-like" pattern. In the Malesian and Pacific genus *Serianthes,* which has a large number of very restricted endemics, almost every island appears to have its own characteristic type of areole outline (Nielsen et al., 1983b) superimposed on a constant exine structure.

DISPERSAL UNIT

The most frequent pollen unit in the Leguminosae is the single grain, and the available pollen fossil record indicates that this type is basic in the three subfamilies. Tetrads (tetrahedral tetrads) are extremely rare in the Caesalpinioideae, occuring only in *Afzelia,* one species of *Bauhinia,* and the species of the genus *Diptychandra* (Erdtman, 1969; Guinet, 1965; Schmitz, 1973; Senesse, 1976; Ferguson, 1987), and are associated with a columellate infratectal exine structure and compound equidistant apertures (three equatorial colpori). Tetrads occur much more frequently in the Mimosoideae; Guinet (1981b) lists the genera where these occur. Generally the occurrence of tetrads is generically or specifically constant when associated with a columellate structure and compound apertures. Polyads occur commonly in the Mimosoideae. They are absent from the Caesalpinioideae. Both tetrads and polyads are lacking from the Papilionoideae.

When the pollen is compound, the morphological progression follows an arithmetic series in which the constant set is the cluster of grains issued from the meiotic tetrad and the variable set thereafter being dependent on the number of sporogenous cells remaining together. Using both the fossil record and the comparative morphology of extant species, the general sequence appears to be:

(4) → 1 (tetrad stage→single grain); in most Leguminosae.
4 × 1: tetrad (stable tetrads in several genera of the Mimoseae tribe (Mimosoideae); in a few Caesalpinioideae.
4 × 2: 8 grain polyads; most frequent in the Mimoseae but recurring in *Acacia* (Acacieae) and *Calliandra* (Ingeae).
4 × 3: 12 grain polyads; in Mimoseae, Acacieae, rarely in Ingeae.
4 × 4: 16 grain polyads; the most common type in the Mimosoideae.
4 × 5: Ingeae: *Chloroleucum.*
4 × 6: in *Tetrapleura* (Mimosae) and again in *Pithecelobium* (Ingae).
4 × 7: Ingeae: several species of the genus *Albizia.*
4 × 8: frequent in the Ingeae (*Albizia* and included genera: *Cathormion, Samanea, Pseudomanea, Macrosamanea, Enterolobium*) but also in *Parkia* (Parkieae).
4 × 9: in *Dichrostachys* (Mimoseae).
4 × 10: in *Leucaena* (Mimosae).
4 × 11: in a few *Acacia* species (Acacieae).
4 × 12: in a few *Acacia, Albizia, Affonsea* (Acacieae, Ingeae).
4 × 14: in *Parkia* (Parkieae).

Polyad dissymmetry is frequently, but not always, linked to this increase in the number of associated grains (can be compared with Apocynaceae, Asclepiadaceae, Orchidaceae). Twelve-grain polyads in the genus *Acacia* may be symmetric or dissymmetric. Here, the early physical constraints during the development (the arrangement of the sporogenous cells before meiosis) may have the same importance as the arrangement of the grains at the tetrahedral stage. Highly dissymmetric eight-grain polyads occur in the genus *Calliandra* s.l. and more rarely in *Dichrostachys* and a few species of *Acacia.* Reversions in the sequence of this series occur in some hybrids and in species

regarded to be of hybrid origin (Guinet, 1983; Guinet, 1986), both in the three subgenera of genus *Acacia* and in the genus *Albizia* (Guinet, unpublished). This indicates that the polyad grain number alone cannot be safely used for the establishment of generic delimitations (however, see Niezgoda & Nevling, 1979). This arithmetic ascending series corresponds very roughly with a change in infratectal exine structure together with a change in the apertural type. This agrees, with a few exceptions (e.g., *Parkia*), with the currently accepted tribal sequence in the Mimosoideae, which is mainly based on the variation in the stamen characters. For example, the tribe Ingeae, with many showy stamens fused at their bases in a tube, shows a higher frequency of more than 16-grain polyads, a granular exine infrastructure, and a porate type. In the compound units, the individual grains may be all "free" (morphologically independent) or more or less closely connected by exine bridges or in various other ways. The extreme case is the calymmate type (grains with a common wall) in which the distal tectal parts of each grain are fused (in *Parkia* and species of *Calliandra*). Such fusion may occur only between grains originating from the same meiotic tetrad (acalymmate polyads are made by the cohesion of calymmate tetrads, as, for example, in *Dichrostachys* [Guinet, unpublished]). These can be compared with the powdery massulae in the Orchidaceae. More often, all the grains of the same dispersal unit are fused (some species of *Parkia* and of *Calliandra*). Such strong cohesion modes have important and nonreversible consequences, on both the dispersal mode and the apertural system (see p. 87).

The general increase in the number of associated grains per polyad leads to the increase in polyad size (with the exception of *Calliandra*, where species occur with 8-grain, very large polyads). This situation is especially noteworthy in the Ingeae, a tribe which contains most flowers adapted to pollination by larger-bodied animals (Elias, 1981). The same trend occurs in the Parkieae in the genus *Parkia*, whose natural affinities are perhaps debatable (Sorsa, 1969; Elias, 1981), but at least indicating that Bentham was right in separating *Parkia* and *Pentaclethera* into two distinct tribes.

IV. Interdependence of the Pollen Characters

As discussed above, exine structure is the most important pollen character for phylogenetic interpretations in the Leguminosae. The link between exine infratectal structural characters and apertural types was first established in the genus *Acacia* by Guinet & Lugardon (1976). In this genus, the apertures are compound (colpori) when the exine is columellar. When granules occur, only pores are differentiated. The same interdependence between exine structure and apertures has been demonstrated by Stainier & Horvat (1978), Horvat & Stainier (1979, 1980), and Maréchal et al., (1978) in the *Phaseolus–Vigna* complex (Papilionoideae) and by Ferguson & Skvarla (1981) in other members of the Papilionoideae, particularly in the Phaseoleae and Psoraleeae (Ferguson & Skvarla, 1983). It is clear that this interdependence is not straightforward and there are many exceptions. However the "columellar" structure at present is only very broadly defined, and we emphasize that in many cases the use of the term "columellae" in a strict sense would considerably reduce the number of "columellar" taxa. Many transitions between the columellar/colporate types and the granular/porate types have been described (Guinet & Lugardon, 1976; Stainier & Horvat, 1978; Maréchal et al., 1978; Ferguson & Skvarla, 1983; Ferguson, 1984), showing that when columellae and granules are associated in a single infratectal layer, and/or the granules are radially ordered, the aperture is either colporate or porate (Stainier & Horvat, 1978; Maréchal et al., 1978). The reason for such an association between exine and apertural characters is unknown. The replacement of colpi by pores when the exine infrastructure is granular suggests that specialized harmogathic devices (colpi) are unnecessary when this granular type of exine structure occurs. Perhaps this is due to its physical properties. This would perhaps explain why the granular exine structure is completely independent of the pollination modes in the angiosperms. Crane (1986) suggested that colpi generate outward bending during harmomegathic movements and that the tectatecolumellate arrangement is resistant to tensile forces. In contrast, in unspecialized porate

grains, the only option for harmomegathic movement is for the wall to buckle inward. Crane suggested that in such situations a granular infratectum is good for resisting compressive forces.

This interdependence is by itself of primary phylogenetic importance (see Table 2). It appears very frequently in the Mimosoideae, a subfamily where the granular infrastructure and the porate type are predominant; here, the change from colporus to porus occurs abruptly without morphological transitions (no short colpi with rounded ends have been observed in the subfamily). The exine/aperture association is again very frequent in the Papilionoideae, with apparently more numerous "mixed" exine structures (for example in Phaseoleae). The genera *Phaseolus* and *Vigna* (Papilionoideae) do not hybridize (Stainier & Maréchal, pers. comm.), but some *Vigna* species have pollen characters with a columellar infratectal structure and colporate apertures. These must be interpreted as having ancestors in the Phaseoleae.

In *Bauhinia* s.l. (Ferguson & Pearce, 1986) various Phaseoleae, particularly in the subtribe Diocleinae (Kavanagh & Ferguson, 1981) and in *Macrotyloma* (Ferguson, 1981), transitions between colpori and pori in the form often of short colpi occur and these are linked with a change in the nature of the infratectal structure. Soladoye & Crane (1985) have suggested, from comparative pollen morphology of the genus *Baphia* (Papilionoideae), that the change in the timing of the exine developmental events may be involved. However, such a change has occurred in a repetitive manner (± 15 genera) in the Leguminosae and in unrelated groups.

The absence or the extreme reduction of the endexine in both Caesalpinioideae and Papilionoideae results in the loss of a typologically distinct internal opening—endoaperture. This is well documented in the Papilionoideae (Ferguson & Skvarla, 1981; Ferguson, 1984). At the other extreme the great reduction of the ectexine may lead to a porate type by "fading" of the colpus of a compound aperture, as seen in *Erythrina* (Ferguson & Skvarla, 1981). Guinet (1981a) has suggested that some apertural characters (colporus versus porus) are directly dependent on the available amount and distribution of ectexine on a pollen grain. This may be true even if the general balance in the exine layers and their specific physical properties confer different responses to forces generated in harmomegathic movements (Heslop-Harrison, 1979; Denizot, 1985; Soladoye & Crane, 1985). It is clear that there is not a very close interdependence between exine structure and external sculpturing in Leguminosae. Frequently, but not always, the diversity of sculpture is associated with a greater development of the ectexine, which in turn is generally linked with an increase in pollen size. This occurs quite independently of variation in the other layers of the exine. However, it is emphasized that exines with a fine reticulate ornamentation and interpreted as the basic type in the family have a columellar structure.

V. Patterns of Change

The main phylogenetically useful pollen characters can be seen in the fossil record. Although the existing fossil record is utterly insufficient for so large a family, several general trends can be recognized. Other trends have been inferred from comparative morphology of the extant species and broadly correlate with the current classification of legumes that is mainly based on a wide range of other characters.

Fossil pollen record

The fossil record (Table 2, Fig. 1), as presently known, indicates three major phases in the main pollen diversification in the family.

1. An initial radiation, showing the establishment of the basic pollen types—early single grain differentiation. The pollen and macro-fossil record indicates that during this interval of time (from Maastrichtian to Lower Eocene, 69–55 Ma) at least two subfamilies, Caesalpinioideae and Mim-

osoideae, had differentiated. The Caesalpinioideae appear the oldest and the most diverse (Muller, 1981, 1984), and the subfamily Papilionoideae has been recognized from floral remains, although the pollen is unknown (Crepet & Taylor, 1985). The genera and species recorded in the Maastrichtian to the Lower Eocene interval all have their pollen in single grains, columellar infratectal structure, and a compound aperture (colporus). The *Sindora* type, related to the present genus *Sindora* (Van Campo, 1963), is the most distinct because of paired endoapertures in the colpi, but the symmetry of the grains is similar to the other tricolporate pollen grains found in the fossil record during the same interval of time. Increase in the number of the endoapertures while the ectoaperture number remains constant is perhaps surprising for this early stage in the Leguminosae. However, a comparable situation occurs at about the same time in other angiosperm families, for example Olacaceae (see Muller, 1981). Although there is no fossil record, this situation probably recurs at a later stage in morphologically advanced genera, for example in the Papilionoideae *(Dumasia, Aeschynomene).* In all the genera recorded, the internal aperture is circular (pore), suggesting that the transverse elongation and diverse modifications in the endoaperture shape—very frequent in the Papilionoideae, very rare in the Caesalpinioideae, and absent in the Mimosoideae—are advanced characters.

2. During the Eocene (55–40 Ma), the change towards compound grains and the predominant differentiation of these forms appears to have taken place. Simultaneously, the granular infratectal exine structure developed. The association of a columellar infrastructure with compound grains is known from the Middle Eocene. Permanent and stable (species-constant) tetrads with three equatorial colpori have been found in the American fossil genus *Eomimosoidea,* whose main pollen characteristics (except exine ornamentation) also occur in the extant African monotypic genus *Fillaeopsis* (Mimosoideae) that is recorded from the Oligocene onwards. In the Upper Eocene, the genus *Parkia* (Mimosoideae) appears with a columellar infratectal exine structure but here associated with simple pores. The genus shows in different species the two exine structures (Guinet, 1981b; Feuer et al., 1985). The fossil species has been related to *Parkia ulei* (Guinet & Salard-Cheboldaeff, 1975), an extant neotropical insect-pollinated species which has the lowest number of flowers per capitulum in the genus (Hopkins, 1984) and 16-grain polyads (in the present species, the polyad grain number varies from 16 to 56).

3. Oligocene onwards (39– Ma). Very few important pollen morphological changes seem to appear from the Oligocene. Further differentiation results from selection from the available pollen characters established in the second period, which appear to be the most easily modified types. In this period differentiation of pseudocolpi (the extra-porate type) is first recorded (see Guinet & Salard-Cheboldaeff, 1975; Guinet et al., 1986). On the whole the Oligocene is marked by an important radiation of the genera and species of the Mimosoideae with a granular infratectal exine structure, associated with the pluriporate type (nonequidistant pores), a character association that presently predominates in the Mimosoideae–Acacieae and –Ingeae. The only remarkable morphological innovations are in the "genus" *Calliandra,* with the occurrence of two unrelated changes. The first is a differentiation of dissymmetric lens-shaped outgrowths of the tectum in 16-grain polyad neotropical species (*Zapoteca;* Hernández, 1986). The second is a differentiation of a single grain within an 8-grain polyad type. The taxonomy of this group is complex and unresolved.

Thus, the fossil record as presently known correlates with the coherent and significantly linked changes in many pollen characters recognized on the basis of comparative palynology. The only pollen morphological features about which the fossil record provides no useful data is the ornamentation of the exine.

POLLEN GRAIN NUMBER

The fossil record indicates an evolutionary change from single grains to tetrads and to polyads. When the pollen is compound, there is a regular increase in the number of associated grains, and

very frequently linked to this is a change of exine structure followed by a change of apertural type. This occurs particularly in the tribe Mimoseae, where the colporate-columellar compound type of pollen grain is replaced later by the porate-granular one (Guinet et al., 1986). The increase in the number of associated grains per polyad occurs in parallel in the main tribes of the Mimosoideae (e.g., *Parkia* (Parkieae), *Piptadenia,* and related genera [Mimoseae]) and in the tribes Acacieae and Ingeae. However, in the two latter tribes this trend, irrespective of change in other pollen characters, is likely to have been easily reversed (Guinet, 1986; Nielsen et al., 1984a). The African species *Acacia laeta* (12-grain polyads) appears to be of hybrid origin (Elamin, 1976). Its putative parents, whatever they are (Elamin, 1976; Brenan, 1955), have 16-grain polyads. African species have polyads with 16 or more grains, but hybrids may have fewer-grained polyads. The Australian species *Acacia baueri* (pollen sometimes in tetrads) cannot be viewed as basic even in the subgenus Phyllodineae. This reduction in polyad grain number, frequent in fertile hybrids, suggests the important and still insufficiently known role of hybridity in many groups of the Mimosoideae.

The general increase in polyad grain number and, as a consequence, the increase in polyad size have important biological consequences, because polyad grain number and generally polyad size are linked to stigma diameter (Niezgoda & Nevling, 1979; Elias, 1974; Kenrick & Knox, 1982; Guinet, 1986) and stigma shape (Hernández, 1986). Such a close correlation must be regarded as an increasing functional specialization (Knox, 1984) as well as an isolating factor favoring speciation by limiting improper pollen transfer (Guinet, 1986). On the other hand, a higher polyad grain number increases the reproductive capacity of species, as polyad grain number and maximum seed number are correlated (Knox & Kenrick, 1983). A macromorphological consequence is that the form of the pod is partially due to the number of developed seeds and also related to the polyad grain number. In the Ingeae, the genus *Archidendron,* and allied genera with a constant polyad grain number, reproductive capacity is increased by an increase in carpel number (Nielsen et al., 1984b). Functional considerations such as these may contribute to the understanding of the geographically restricted speciation patterns and the role of some species groups in tropical ecosystems, e.g., the intense speciation of the Australian *Acacia* species in SSW Australia (8-grain and 12-grain species in SSW Australia and the predominance of the 28–32-grain polyads in the neotropical species of the genus *Albizia,* as well as the restricted geographic development of the 8-grain polyad species with a long "tail cell" in the genus *Calliandra* (Amazonian Basin). Another example is that a lower reproductive capacity may occur in some species or species groups (e.g., *Acacia* and *Calliandra*) due in part to a low grain number in the polyad. When this occurs it would tend to reduce at least the number of conspecific individuals in some vegetation types and thus tend to increase species number.

EXINE STRUCTURE AND APERTURES

A parallel and frequently independent change occurs in the exine that indicates a general progression from a predominantly columellar infratectal structure occurring in the core groups of the three subfamilies that have single tricolporate grains. This columellar structure is maintained in tetrahedral tetrads that consist of grains with three equatorial colpori. It occurs in a few Caesalpinioideae and Mimosoideae, generally in monotypic genera or genera with few species, as, for example, *Fillaeopsis, Schleinitzia,* and an unnamed genus from Madagascar. These characters are very rarely associated within polyads (*Acacia* subg. *Acacia* and a few species of *Calliandra*). From these types we can interpret the granular infratectal structure associated with porate apertures as being generally derived in Leguminosae.

Other modifications of the exine are equally important and must occur either isolated or in addition to the change in infratectal structure. These are:

1. The very weak differentiation of the endexine or its absence (at least as a continuous layer). We interpret such a reduction or absence as a derived condition, resulting in a derived colpate pollen

type. Trends towards this thinning of the endexine at least in the equatorial area are correlated, as expected, with a diffuse endoaperture. This occurs in the predominantly New World tribes Aeschynomeneae, Amorpheae, and Adesmieae of the Papilionoideae. The direct link between the lack of endexine and simplification of the apertural system is not restricted to the Leguminosae (Walker & Doyle, 1975; Lobreau-Callen, 1977; Van Campo, 1980) but occurs more frequently in relatively advanced angiosperms. The opposite trend is a strong thickening of the endexine, most frequently associated with a reduction or a loss of the foot layer. Where this occurs the apertural type is unchanged, being colporate in Papilionoideae and porate in Mimosoideae, when the infratectal exine structure is unchanged. When there is a tendency for shortening of the colpi and some apertural change towards pory, an associated change toward a granular infratectal structure often occurs (e.g., Desmodieae [Ferguson & Skvarla, 1979] and Phaseoleae, discussed above).

2. Differentiation of the ectexine, excluding the infratectal structure, is more complex because the distribution of the ectexine over the whole volume of the pollen grains is very variable. There is a tendency for greater ectexine differentiation in the equatorial area, frequently associated with a reduction in the endexine and the development of a pseudo-operculum (i.e., a local thickening of the colpus membrane as shown by Roland, 1966) or an operculum (endexine present under the opening). Pseudo-opercula are very frequent in the Papilionoideae but also occur in the Caesalpinioideae and the Mimosoideae. These pseudo-opercula or local thickenings are frequently developed only in the equatorial areas of the grains in the center of each aperture. Equatorial continuity of the colpal and intercolpal ectexines leads to the differentiation of "semicolpi" in some species of *Leucaena*, *Prosopis*, and *Neptunia* in the Mimosoideae. In *Sindora* (Caesalpinioideae) (Van Campo, 1963) and in *Aeschynomene* (Papilionoideae) (Guinet & Ferguson, unpublished) thickening and fusion of the ectexine at the equator leads to the differentiation of the polaro-aperturate type (Van Campo, 1963) (endoapertures in two parallel rows, which are in turn parallel to the equator). We interpret this type as derived from the primitive tricolporate form.

INCREASE IN APERTURE NUMBER

Increase in aperture number on the equatorial plane is a characteristic tendency in the Papilionoideae but is apparently rare or absent from the two other subfamilies; in the Caesalpinioideae the genera *Ceratonia* with 4 colpi and *Bauhinia* with 5–7 colpi in some species are exceptional. Nonequatorial increase in aperture number occurs in all three subfamilies but is much more frequent in the Mimosoideae, where it is almost exclusively associated with pory, even when the distribution of the exine is dissymmetric (compound grains). The pores in this group cannot be equidistantly spaced due to internal constraints.

CHANGES IN SCULPTURE

These changes appear to be related to changes in external factors. This is indicated by (1) the absence of unambiguous trends in ornamentation in the fossil record and (2) the recurrence of similar sculpture unrelated to the changes in other pollen characters in taxa in the three subfamilies. Only the fine reticulate ornamentation, which is the basic type and one of the less frequent, is regularly linked to the columellar infratectal exine structure. Documented examples of the link between sculpture and external factors are known in the three subfamilies (Caesalpinioideae, Ferguson & Pearce, 1986; Mimosoideae, Guinet, 1986; Hernández, 1986; and Papilionoideae, Ferguson & Skvarla, 1983; Ferguson, 1984; Hemsley & Ferguson, 1985). Usually the most apparent link is with pollination modes and their diversity (Crepet, 1984). Here it is not surprising to find repetitive correlations between pollen sculpture and flower and/or inflorescence morphology, e.g., zygomorphy and transference of the attractive role to the inflorescence rather

than to individual flowers, which suggests strongly correlated characters. Future pollen morphological and ecological studies will most probably demonstrate that this link is even more frequent than presently recognized. In the genus *Baikiaea* in the Caesalpinioideae (Ferguson, 1987), broader reticulate exine surfaces are correlated with showy flowers. A similar situation is found in the genus *Dunbaria* (Papilionoideae), but here ecogeographic factors are also related (Thuan, 1973). All these examples in the three subfamilies afford confirmation of the frequent size relationships between pollinators, flowers, and pollen (Lee, 1978). In the genus *Acacia,* subg. Phyllodineae (Mimosoideae) the changes in the size of the supratectal reticulum correlate closely with the main Australian climatic zones and are unrelated to the apparently uniform floral characters. In *Acacia* a broad reticulum is dominant in tropical wet areas, and its reduction under colder conditions must be strongly suspected (Guinet, 1986). The genera *Phaseolus* (more temperate than tropical, small-reticulate exine) and *Vigna* (more tropical than temperate, broad-reticulate exine) provide other examples in which temperature variations probably influence pollen morphology (see also apertures, p. 89).

Correlations between exine ornamentation and external factors also occur in the Papilionoideae–Desmodieae (Ohashi, 1971, 1973), in which the reduction of the reticulum lumina size is associated with the reduction (compact) of the inflorescence and seasonally colder climatic conditions, but not necessarily with changes in floral characters. In the Papilionoideae–Diocleinae, the taxa with reticulate sculpture are generally savannah species, while the micropunctate ones are generally forest-margin species (Kavanagh & Ferguson, 1981). Infraspecific pollen variation is frequently linked to the size of the species area, and then in turn to ecological diversity (e.g., *Acacia* [Guinet et al., 1980; Guinet, unpublished], *Archidendron* [Nielsen et al., 1984b], and *Erythrina* [Hemsley & Ferguson, 1985]). It has been observed (Clarke, 1975) that the growth under unusual ecological conditions increases the amplitude in the variation of pollen characters. However, climatic changes interact with many other factors, from changes in pollinator frequency (Heinrich & Raven, 1972) to changes in soil mineral nutritive elements (see Muller, 1979), so that only experimental studies such as those of Hebda & Lott (1973) can provide more precise conclusions.

Those kinds of environmentally induced changes may also have important consequences; for example, the change from single to compound grains is evidently controlled by the number of mitotic divisions of the sporogenous cells preceding meiosis (Kenrick & Knox, 1982). The mechanism of control is insufficiently known (Takahashi & Sohma, 1984) but has the important consequences that the polyad functions as a single harmomegathic unit (McGlove, 1978), due to both the unequal sporopollenin distribution on each grain and the degree of cohesion of the grains. In the acalymmate polyads, every grain may still function on its own and maintain its proper harmomegathic system. The occurrence of pseudocolpi and a thicker tectum on only the eight central grains of a 16-grain polyad in several Australian species of the genus *Acacia* in subgenus Phyllodineae may be related to the maintenance of this harmomegathic independence. In some species of *Parkia* and *Calliandra* that have calymmate polyads, there is a striking "clustering effect" where adjacent pores on the angles of each grain are fused. This results in three pores having a single ectoaperture and might be compared with the situation in Ericaceae where there are two pores with a single colpus. A similar situation occurs in Asclepiadaceae (Linskens & Suren, 1969).

The high degree of heteromorphy in the polyads of some Mimosoideae (in some Madagascan *Dichrostachys,* some southwestern Australian species of *Acacia,* and in species of *Calliandra* with 8-grain polyads) must be interpreted as the morphological manifestation of a very strong interrelationship with specialized pollinators. High polyad dissymmetry is associated with the occurrence of a mucilage coating on the top of a single specialized grain in some *Calliandra* species or with dark-colored (brown-red) polyads both in this genus and *Serianthes* (Nielsen et al., 1983a) and in many species of the genera *Parkia, Dichrostachys, Affonsea,* and *Inga* (Guinet, unpublished). The pollen units (150–350 μm) are also remarkably large. This suite of characters probably indicates the transference of the attractive role from individual flowers to pollen and probably also to stamens, which are frequently red and showy in the Ingeae. This character syndrome appears much more

frequent in the predominantly or exclusively bird/bat-pollinated species (Dressler, 1971, but see Inouye, 1975).

Conclusions

Divergent evolution, as indicated by pollen changes, is prevalent in all groups of the Leguminosae but, as expected in so large a family, it is generally expressed in very diverse ways. This diversity increases the difficulties in attaining a general understanding of the broad trends at the family level. We have attempted (Fig. 2) to set up a general model applicable to all the major groups and irrespective of the remarkable particularities of many of them. Such a model is tentative, and there is a need to compare pollen trends with hypotheses of phylogeny based on other characters. The most outstanding feature in the Leguminosae is perhaps the great development and diversification of an unusual pollen type in the angiosperms, the compound grain. Although this type occurs very widely, from the Annonaceae to the Orchidaceae there is no family in which these features exhibit such a high degree of diversity. However, in this paper we have avoided placing too much emphasis on the diversity in compound grains and the characters directly linked with these structures. We have tried to give more emphasis to the trends that are common in the pollen morphology of the whole family, believing that such an approach will be of greater assistance in deciding relationships.

Many of these common trends are those currently recognized throughout the angiosperms, such as increase in aperture number, change towards pory, and increase in pollen size (Van Campo, 1946, 1948, 1976; Walker & Doyle, 1975), which is clearly linked in the Leguminosae with pollination syndromes. In some cases an opposite interpretation may be possible or an opposite trend must have occurred. We have attempted, with emphasis on both the increasing fossil record and recent information drawn from comparative morphology of the extant taxa, to decide whether a character must be primitive or derived, and if derived, derived from what? Also we have tried to show which characters change alone and which change in association with others. It is of great interest in general systematics to know which pollen characters are linked, even if in most cases we are unable to say why, because such links increase, perhaps sometimes too much, overall similarity or dissimilarity between taxa. The importance of the correlation between exine structure with many characters of the apertures is emphasized. These correlations in turn correspond with established taxonomic groupings at the generic and subgeneric levels and offer better guides to phylogenetic affinities. Study of pollen characters in this way may lead to a more accurate recognition of the differentiation patterns along different lines. These patterns are probably related to strong and long selection factors acting in a constant direction. This should result in the arrangement of the systematic units in the most probable natural sequence. However, for such an aim all pollen characters would need to have the same systematic significance or value. But all pollen characters do not have the same significance. Rowley (1981) has suggested that characters of the exine structure are comparatively more conservative. Some pollen characters are more readily susceptible to change in response to variation in external factors than others. Such characters may easily change within the limits imposed by their structure in response to their variation in time. This would perhaps explain the greater frequency of the repetitive occurrence of superficially similar patterns (convergent sculptural types) most frequently associated with the variation in flower size, color, symmetry, inflorescence types, and stamen number as compared to the frequency of the changes relating to pollen characters. But on the whole, this analysis of the diversity of the pollen characters in the Leguminosae suggests a faster common response to external factors, showing that the pollen characters are no more conservative than others (Heslop-Harrison, 1979; Knox, 1984). Acceptance of this point of view would lead to the development of a new field of research on pollen ecology.

References

Bentham, G. 1865. *In* G. Bentham & J. D. Hooker, Genera Plantarum, Volume 1. Lovell Reeve, London.

Bessedik, M. 1985. Reconstitution des environments Miocènes des régions N.O. Mediterranéenes à partir de la palynologie. Thesis, Montpellier.

Brenan, J. P. M. 1955. Notes on Mimosoideae, I. Kew Bull. 9: 161–192.

_____. 1959. Leguminosae, subfamily Mimosoideae. *In* C. E. Hubbard & E. Milne-Redhead (editors), Flora of Tropical East Africa. Crown Agents, London.

Bronckers, F. 1967. *Phaseolus & Vigna* in palynologie Africaine, VII. Bull. Inst. Fondam. Afrique Noire 29: 471, pls. 141–147.

Burns-Balogh, P. 1983. A theory on the evolution of the exine in Orchidaceae. Amer. J. Bot. 70: 1304–1312.

_____ & V. A. Funk. 1986. A phylogenetic analysis of the Orchidaceae. Smithsonian Contr. Bot. 61: 1–79.

Caccavari, M. A. 1985. Granos de polen do Leguminosas de la Argentina IV. Genera *Mimosa*. Bol. Soc. Argent. Bot. 24: 151–167.

Clarke, G. C. S. 1975. Irregular pollen grains in some *Hypericum* species. Grana 15: 117–125.

Cocucci, A. & W. A. Jensen. 1969. Orchid embryology: pollen tetrads of *Epidendrum scutella* in the anther and on the stigma. Planta 84: 215–229.

Coetzee, J. A. 1955. The morphology of *Acacia* pollen. S. African J. Sci. 52: 23–27.

Cowan, R. S. 1975. A monograph of the genus *Eperua* (Leguminosae–Caesalpinioideae). Smithsonian Contr. Bot. 28: 1–45.

Crane, P. R. 1986. Form and function in wind dispersed pollen. Pp. 179–202 *in* S. Blackmore & I. K. Ferguson (editors), Pollen and Spores: Form and Function. Academic Press, London.

Crepet, W. L. 1984. Advanced (constant) insect pollination mechanisms: patterns of evolution and implications vis-à-vis angiosperm diversity. Ann. Missouri Bot. Gard. 71: 607–630.

_____ & D. W. Taylor. 1985. The diversification of the Leguminosae: first fossil evidence of the Mimosoideae and Papilionoideae. Science 228: 1087–1088.

_____ & _____. 1986. Primitive mimosoid flowers from the Paleocene–Eocene and their systematic and evolutionary implications. Amer. J. Bot. 73: 548–563.

Daghlian, C. P., W. L. Crepet & T. Delevoryas. 1980. Investigations of Tertiary angiosperms: a new flora including *Eomimosoidea plumosa* from the Oligocene of eastern Texas. Amer. J. Bot. 67: 309–320.

Denizot, J. 1985. Recherches ultrastructurales sur les parois des spores inaperturées de deux Marchantiales en cours de germination. 100e Congr. Natl. Soc. Savantes, Montpellier, Sci. Fasc. 2: 187–201.

Dickison, W. C. 1981. Evolutionary relationship of the Leguminosae. Pp. 35–54 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Dressler, R. L. 1971. Dark pollinia in hummingbird-pollinated orchids, or do hummingbirds suffer from strabismus? Amer. Naturalist 105: 80–83.

Elamin, H. M. 1976. *Acacia laeta* (R. Br. ex.) Benth., considered as a species of hybrid origin. Sudan Silva 21: 14–23.

El Baba, J. 1982. Contribution à l'étude du genre *Trifolium* L. au Liban. Thesis, Aix-Marseille.

Elias, T. S. 1974. The genera of Mimosoideae (Leguminosae) in the southeastern United States. J. Arnold Arbor. 55: 67–118.

_____. 1981. Mimosoideae–Parkieae. P. 153 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Erdtman, G. 1947. Suggestions for the classification of fossil and recent pollen grains and spores. Svensk Bot. Tidskr. 41: 104–114.

_____. 1969. Handbook of Palynology. Munksgaard, Copenhagen.

Ferguson, I.K. 1978. A note on the pollen morphology of the genus *Cranocarpus* Bentham (Leguminosae). Bradea 2: 269–272.

_____. 1980a. The pollen morphology of *Ceratonia* (Leguminosae–Caesalpinioideae). Kew Bull. 35: 273–277.

_____. 1980b. Quelques remarques sur l'ultrastructure du pollen deux espèces de la sous-famille des Papilionoideae (Leguminosae) en relation avec le microenvironment (hygrométrie et hydrodynamique). Mem. Mus. Natl. Hist. Nat., Sér. B. Bot. 27: 45–50.

_____. 1981. The pollen morphology of *Macrotyloma* (Leguminosae: Papilionoideae: Phaseoleae). Kew Bull. 36: 455–461.

———. 1984. Pollen morphology and biosystematics of the subfamily Papilionoideae (Leguminosae). Pp. 377–394 *in* W. F. Grant (editor), Plant Biosystematics: Forty Years Later. Academic Press, Canada.

———. 1986. The role of pollen morphology in plant systematics. Ann. Assoc. Palinol. Lengua Espanola 2: 5–18.

———. 1987. A preliminary survey of the pollen exine stratification in the Caesalpinioideae. Pp. 355–385 *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

——— & K. J. Pearce. 1986. Observations on the pollen morphology of the genus *Bauhinia* (Leguminosae: Caesalpinioideae) in the Neotropics. Pp. 283–296 *in* S. Blackmore & I. K. Ferguson (editors), Pollen and Spores: Form and Function. Academic Press, London.

——— & J. J. Skvarla. 1979. The pollen morphology of *Cranocarpus martii* Bentham (Leguminosae: Papilionoideae). Grana 18: 15–20.

——— & ———. 1981. The pollen morphology of the subfamily Papilionoideae (Leguminosae). Pp. 859–896 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

——— & ———. 1982. Pollen morphology in relation to pollinators in Papilionoideae (Leguminosae). Bot. J. Linn. Soc. 84: 1401–1408.

——— & ———. 1983. The granular interstitium in the pollen of the subfamily Papilionoideae (Leguminosae). Amer. J. Bot. 70: 1401–1408.

——— & R. Strachan. 1982. Pollen morphology and taxonomy of the tribe Indigoferae (Leguminosae: Papilionoideae). Pollen & Spores 24: 171–210.

Feuer, S. 1987. Combined cryo- and thin sectioning (TEM) in the elucidation of mimosoid tetrad/polyad ultrastructure. Rev. Palaeobot. Palynol. 52: 367–374.

———, C. J. Niezgoda & L. J. Nevling. 1985. Ultrastructure of *Parkia* polyads (Mimosoideae: Leguminosae). Amer. J. Bot. 72: 1871–1890.

Graham, A. & G. Barker. 1981. Palynology and tribal classification in the Caesalpinioideae. Pp. 801–834 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———, ——— & M. Freitas da Silva. 1980. Unique pollen types in the Caesalpinioideae (Leguminosae). Grana 19: 79–84.

Guinet, Ph. 1965. Remarques sur les pollens composés à parois internes perforées. Pollen & Spores 7: 13–18.

———. 1969. Les Mimosacées. Étude de palynologie fondamentale, corrélations, evolution. Trav. Sect. Sci. Tech. Inst. Fr. Pondichéry 9: 1–293.

———. 1978. Biologie évolutive dans le genre *Acacia:* arguments palynologiques. Mém. Trav. Ecole Prat. Hautes Études: Inst. Montpellier 4: 41–51.

———. 1981a. Comparative account of pollen characters in the Leguminosae. Pp. 789–799 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———. 1981b. Mimosoideae: the characters of their pollen grains. Pp. 835–857 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———. 1983. Distribution of the pollen characters in some hybrids and in some suspected hybrids in the genus *Acacia*. Bull. Int. Group Study Mimosoideae 11: 25–28.

———. 1986. Geographic patterns of the main pollen characters in genus *Acacia* (Leguminosae), with particular reference to subgenus Phyllodineae. Pp. 297–311 *in* S. Blackmore & I. K. Ferguson (editors), Pollen and Spores: Form and Function. Academic Press, London.

——— & M. Bessedik. 1984. Présence du genre *Prosopis* (Leguminosae–Mimosoideae) à l'Aquitanien basal dans l'Aude (Languedoc-France). Pollen & Spores 26: 101–108.

——— & C. Gruas-Cavagnetto. 1986. Présence du genre *Neptunia* (Leguminosae–Mimosoideae) à l'Eocene moyen dans la Bassin tertiaire au nord de la Baie de Seine. Pollen & Spores 28: 167–176.

——— & A. Le Thomas. 1973. Interprétation de la répartition dissymétrique des couches de l'exine dans les pollens composés. Conséquences relatives à la notion d'aperture. Compt. Rend. Hebd. Séances Acad. Sci. 276: 1545–1548.

——— & B. Lugardon. 1976. Diversité des couches de l'exine dans le genre *Acacia* (Mimosaceae). Pollen & Spores 18: 483–511.

——— & M. Salard-Cheboldaeff. 1975. Grains de pollen du Tertiaire du Cameroun pouvant être rapportés aux Mimosacées. Boissiera 24: 21–28.

———, N. El Sabrouty, H. Soliman & A. Omran. 1987. Étude des caractères du pollen des Legumineuses–

Mimosoideae trouvées dans les sédiments tertiaries du Nord-Ouest de l'Egypte. Mém. Trav. Ecole Prat. Hautes Études Inst. Montpellier 17: 159–171.

_____, J. Vassal, C. S. Evans & B. R. Maslin. 1980. *Acacia* (Mimosoideae): composition and affinities of the series Pulchellae Bentham. Bot. J. Linn. Soc. 80: 53–68.

Hebda, R. J. & J. N. A. Lott. 1973. Effects of different temperatures and humidities during growth on pollen morphology; an SEM study. Pollen & Spores 15: 563–571.

Heinrich, B. & P. H. Raven. 1972. Energetics and pollination ecology. Science 176: 597–602.

Hemsley, A. J. & I. K. Ferguson. 1985. Pollen morphology of the genus *Erythrina* (Leguminosae: Papilionoideae) in relation to floral structure and pollinators. Ann. Missouri Bot. Gard. 72: 570–590.

Hernández, H. L. 1986. *Zapoteca:* a new genus of neotropical Mimosoideae. Ann. Missouri Bot. Gard. 73: 755–763.

Heslop-Harrison, J. 1979. Pollen walls as adaptive system. Ann. Missouri Bot. Gard. 66: 813–829.

Hooghiemstra, H. 1984. Vegetational and climatic history of the high plains of Bogota, Colombia, a continuous record of the last 3.5 million years. Diss. Bot. 79: 1–368.

Hopkins, H. C. 1984. Floral biology and pollination ecology of the neotropical species of *Parkia*. J. Ecol. 72: 1–23.

Horvat, F. & F. Stainier. 1979. L'étude de l'exine dans le complexe *Phaseolus-Vigna* et dans les genres apparentés. III. Pollen & Spores 21: 17–30.

_____ & _____. 1980. L'étude de l'exine dans le complexe *Phaseolus-Vigna* et dans des genres apparentés. IV. Pollen & Spores 22: 139–172.

Ikuse, M. 1956. Pollen Grains of Japan. Hirokawa Publ., Tokyo.

Inouye, D. W. 1975. Why don't more hummingbird-pollinated flowers have dark-coloured pollen? Amer. Naturalist 109: 377–378.

Kavanagh, T. A. & I. K. Ferguson. 1981. Pollen morphology and taxonomy of the subtribe Diocleinae (Leguminosae: Papilionoideae: Phaseoleae). Rev. Palaeobot. Palynol. 32: 317–367.

Kenrick, J. & B. R. Knox. 1982. Function of the polyad in the reproduction of *Acacia* (Leguminosae, Mimosoideae). Ann. Bot. (London) 50: 721–727.

Knox, B. R. 1984. The Pollen Grain. Pp. 197–271 *in* B. M. Johre (editor), Embryology of Angiosperms. Springer-Verlag, Berlin.

_____ & J. Kenrick. 1983. Polyad function in relation to breeding systems of *Acacia*. Pp. 411–417 *in* D. L. Mulcahy & E. Ottaviano (editors), Pollen: Biology and Implications for Plant Breeding. Elsevier Science Publ., Amsterdam.

Larsen, K. & S. S. Larsen. 1984. Note on *Zenia* (Caesalpiniaceae) and its pollen morphology. Bol. Soc. Brot. 53: 809–812.

Larsen, S. S. 1975. Pollen morphology of Thai species of *Bauhinia* (Caesalpiniaceae). Grana 14: 114–131.

Lee, S. 1978. A factor analysis study of the functional significance of angiosperm pollen. Syst. Bot. 3: 1–19.

Lewis, G. P. & Ph. Guinet. 1986. Notes on *Gagnebina* (Mimosoideae–Leguminosae) in Madagascar and neighbouring islands. Kew Bull. 41: 463–470.

Linskens, H. F. & M. L. Suren. 1969. Die Entwicklung des Polliniums von *Asclepias curassavica*. Ber. Deutsch. Bot. Ges. 82: 527–534.

Lobreau-Callen, D. 1977. Nouvelle interprétation de l'"ordre" des Célastrales à l'aide de la palynologie. Compt. Rend. Hebd. Séances Acad. Sci. 284: 915–918.

_____ & B. Lugardon. 1983. L'aperture à repli du pollen des Celastraceae. Nat. Monspel. Bot. 23–24: 205–210.

McGlove, M. S. 1978. Pollen wall structure of the New Zealand species of *Epacris* (Epacridaceae). New Zealand J. Bot. 16: 83–89.

Maréchal, R., J. M. Mascherpa & F. Stainier. 1978. Étude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionoideae) sur la base des donnés morphologiques et polliniques, traités par l'analyse informatique. Boissiera 28: 1–273.

Melville, M. 1965. Le pollen des Sapindacées d'Afrique occidentale. Pollen & Spores 7: 465–489.

Muller, J. 1979. Form and function in angiosperm pollen. Ann. Missouri Bot. Gard. 66: 593–632.

_____. 1981. Fossil pollen records of extant angiosperms. Bot. Rev. 47: 1–142.

_____. 1984. Significance of fossil pollen for angiosperm history. Ann. Missouri Bot. Gard. 71: 419–443.

_____ & P. W. Leenhouts. 1976. A general survey of pollen types in Sapindaceae in relation to taxonomy. Pp. 407–445 *in* I. K. Ferguson & J. Muller (editors), The Evolutionary Significance of the Exine. Academic Press, London & New York.

Nevling, L. I. & T. S. Elias. 1970. *Calliandra* pollinia and systematic implications. Amer. J. Bot., Suppl. 59: 753. [Abstract.]

Nielsen, I., Ph. Guinet & T. Baretta-Kuipers. 1983a. Studies in the Malesian, Australian and Pacific Ingeae (Leguminosae–Mimosoideae): the genera *Archidendropsis, Wallaceodendron, Paraserianthes, Pararchidendron* and *Serianthes* (part 1). Bull. Mus. Natl. Hist. Nat., Sect. B, Adansonia 5: 303–329.

———, ——— & ———. 1983b. Studies in the Malesian, Australian and Pacific Ingeae (Leguminosae–Mimosoideae): the genera *Archidendropsis, Wallaceodendron, Paraserianthes, Pararchidendron* and *Serianthes* (part 2). Bull. Mus. Natl. Hist. Nat., Sect. B. Adansonia 5: 335–360.

———, ——— & ———. 1984a. Studies in the Malesian, Australian and Pacific Ingeae (Leguminosae–Mimosoideae): the genera *Archidendropsis, Wallaceodendron, Paraserianthes, Pararchidendron* and *Serianthes* (part 3). Bull. Mus. Natl. Hist. Nat., Sect. B, Adansonia 6: 79–111.

———, ——— & ———. 1984b. The genus *Archidendron* (Leguminosae–Mimosoideae). Opera Bot. 76: 1–120.

Niezgoda, C. J. & L. I. Nevling. 1979. The correct placement of *Albizia carbonaria* Britton. Phytologia 44: 307–312.

———, F. M. Feuer & L. I. Nevling. 1983. Pollen ultrastructure of the tribe Ingeae (Mimosoideae: Leguminosae). Amer. J. Bot. 70: 650–667.

Ohashi, H. 1971. A taxonomic study of the tribe Coronillae (Leguminosae), with a special reference to pollen morphology. J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 11: 25–92.

———. 1973. The asiatic species of *Desmodium* and its allied genera (Leguminosae). Ginkgoana 1: 1–318.

Patel, V. C., J. J. Skvarla & P. H. Raven. 1984. Pollen characters in relation to the delimitation of Myrtales. Ann. Missouri Bot. Gard. 71: 858–959.

———, ———, I. K. Ferguson, A. Graham & P. H. Raven. 1985. The nature of the threadlike structures and other morphological characters in *Jacqueshuberia* pollen (Leguminosae: Caesalpinioideae). Amer. J. Bot. 72: 407–413.

Polhill, R. M. & P. H. Raven (editors). 1981. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

———, ——— & C. H. Stirton. 1981. Evolution and systematics of the Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. Royal Botanic Gardens, Kew.

Roland, F. 1966. Étude de l'ultrastructure des apertures-pollens à pores. Pollen & Spores 8: 409–419.

Ross, J. H. 1977. Caesalpinioideae. Fl. S. Africa 16(2): 1–138. Botanical Research Institute, Pretoria.

Rowley, J. R. 1976. Dynamic changes in pollen wall morphology. Pp. 39–65 *in* I. K. Ferguson & J. Muller (editors), The Evolutionary Significance of the Exine. Academic Press, London & New York.

———. 1981. Pollen wall characters with emphasis upon applicability. Nordic J. Bot. 1: 357–380.

——— & A. O. Dahl. 1977. Pollen development in *Artemisia vulgaris*, with special reference to glycocalyx material. Pollen & Spores 19: 169–284.

Schill, R. & W. Pfeiffer. 1977. Untersuchungen an Orchideenpollinien unterbesonderer beruecksichtigung ihrer feinskulpturen. Pollen & Spores 19: 5–118.

Schmitz, A. 1973. Contribution palynologique à la taxonomie des Bauhinieae (Caesalpiniaceae). Bull. Jard. Bot. Natl. Belgique 43: 369–423.

Senesse, S. 1976. Le pollen des *Afzelia africains* (Leguminosae: Caesalpiniaceae). Adansonia 15: 357–378.

———. 1980. Caesalpiniaceae. Palynologia madagassica et mascarenica. Pollen & Spores 22: 355–423.

Skvarla, J. J. & B. L. Turner. 1966. Systematic implications from electron microscopic studies of Compositae pollen—a review. Ann. Missouri Bot. Gard. 53: 220–256.

Small, E., I. J. Bassett & C. W. Crompton. 1981. Pollen variation in tribe Trigonelleae (Leguminosae) with special reference to *Medicago*. Pollen & Spores 23: 295–320.

Soladoye, M. O. & P. R. Crane. 1985. Systematic palynology of *Baphia* (Sophoreae, Papilionoideae). Grana 24: 145–160.

Sorsa, P. 1969. Pollen morphological studies on the Mimosaceae. Ann. Bot. Fenn. 6: 1–34.

Stainier, F. & F. Horvat. 1978. L'étude de l'exine dans le complexe *Phaseolus–Vigna* et dans les genres apparentés. I. Pollen & Spores 20: 195–214.

——— & ———. 1983. L'étude de l'exine dans le complexe *Phaseolus–Vigna* et dans des genres apparentés. V. Le sous-genre *Sigmoidotropis* (Piper) Verdcourt et *Ramirezella stroboliphera* (Robinson) Rose. Pollen & Spores 25: 5–40.

——— & ———. 1985. Modifications de la structure fine de l'endexine au niveau de l'aperture chez *Phaseolus*

metcalfei Woot. & Standl. et chez *Vigna unguiculata* (L.) Walpers. subsp. *unguiculata* Cv-Gr "biflora." Sci. Géol. Bull. Strasbourg 38: 121–130.

Takahashi, H. & K. Sohma. 1984. Development of pollen tetrads in *Typha latifolia* L. Pollen & Spores 26: 5–18.

Thuan, N. V. 1973. Contribution à l'étude pollinique de genre *Dunbaria* (Papilionoideae). Pollen & Spores 15: 363–384.

Van Campo, M. 1946. Observations sur l'emploi des grains de pollen en phylogénie. Bull. Soc. Hist. Nat. Toulouse 81: 1–4.

_____. 1948. Considérations générales sur l'évolution des grains de pollen. Bull. Soc. Hist. Nat. Toulouse 83: 1–8.

_____. 1963. Quelques réflexions sur les pollens de *Sindora.* Grana Palynol. 4: 361–366.

_____. 1967. Pollen et classification. Rev. Palaeobot. Palynol. 3: 65–71.

_____. 1976. Patterns of pollen morphological variation within taxa. Pp. 125–137 *in* I. K. Ferguson & J. Muller (editors), The Evolutionary Significance of the Exine. Academic Press, London & New York.

_____. 1980. Un test palynologique de la classification de Cronquist-Takhtajan. Abst. 5, Int. Palynol. Conf. Cambridge: 396.

_____ & Ph. Guinet. 1961. Les pollens composés. L'exemple des Mimosacées. Pollen & Spores 8: 201–218.

Walker, J. W. & J. A. Doyle. 1975. The bases of angiosperm phylogeny palynology. Ann. Missouri Bot. Gard. 62: 664–723.

Wodehouse, R. P. 1935. Pollen Grains. McGraw-Hill, New York.

POLLEN, STIGMA, AND STYLE INTERACTIONS IN THE LEGUMINOSAE

S. J. Owens[1] and C. H. Stirton[2]

Abstract

This paper presents an overview of pollen, stigma, and style interactions in the Leguminosae and is based on the following three papers that treat each subfamily separately. It is clear that there are large gaps in our knowledge and that each subfamily has been studied with different emphasis and using different techniques. It is also clear that the bulk of the data has been gathered from a study of mostly advanced tribes or species of immediate economic importance and, in the Mimosoideae, from a relatively few species of *Acacia*. A comparative analysis is therefore difficult. A number of features are unique to the Mimosoideae (e.g., *Acacia*, solid transmitting tissue) and Papilionoideae (e.g., entasis), but it is clear that the Caesalpinioideae show greater diversity and variability in pistil structure, which confirms the belief that they are the most primitive. A summary is provided of the different pistil structures in each of the subfamilies and is cross-referenced to figures in each of the three papers. To facilitate future surveys, a generalized model is presented which highlights the many actual and potential physical and chemical constraints on pollen germination and tube growth that need to be considered in providing data for a full comparative analysis.

I. Introduction

The aims of this paper are to introduce the next three papers, to indicate where there are still gaps in the data, and to highlight data relating principally to phylogenetic or comparative studies. A greater part of the data included in the three papers published herein relating to pollen, stigma, and style interactions has appeared in the literature over the past decade and much of this has emanated from the laboratories of those presenting papers. The work on Papilionoideae by Heslop-Harrison & Heslop-Harrison (1982a, b, 1983, 1984) and the work of Lord and coworkers (e.g., Lord & Webster, 1979; Lord & Heslop-Harrison, 1984; Lord & Kohorn, 1986) are the principal exceptions. The data are by no means exhaustive, however, and it is clear from each paper that not only are there many gaps but each subfamily has been studied with different emphasis.

In the Caesalpinioideae, only 47 from a total of approximately 152 genera (31%) have been examined, in most cases with only one or a few species studied from each genus. This is especially true with regard to the examination of fresh material and to pollen, stigma and style development, and pollen tube growth studies. In the Mimosoideae (± 62 genera), a comprehensive study has focused on *Acacia*, predominantly on those species of *Acacia* found in Australia. In the Papilionoideae (> 450 genera), the genera which have been examined in any depth are predominantly those of advanced tribes with emphasis on cultivated groups.

II. The Pollen

The structure and development of pollen has largely been ignored in the papers on the Caesalpinioideae and Papilionoideae. There appears to be little to add to the recent reviews published, for example, in *Advances in Legume Systematics:* Caesalpinioideae (Graham & Barker, 1981) and

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K.

[2]The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, U.K. Current address: Department of Botany, University of Natal, Box 375, Pietermaritzburg, 3200, Republic of South Africa.

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Papilionoideae (Ferguson & Skvarla, 1981). Ferguson (1987) provides an important survey of pollen exine stratification in the Caesalpinioideae, but much effort might justifiably be placed on pollen development. Pollen in these two subfamilies is uniformly bicellular (Brewbaker, 1967).

Kenrick & Knox (this volume) have reviewed the pollen on Mimosoideae (see also Guinet & Ferguson, this volume). One reason for this review is the widespread occurrence of compound grains, and their obvious significance in the pollen–stigma interaction and pollen–ovule ratios. Kenrick & Knox (this volume) also produce new data, citing changes in the generative cell that occur during development from the female to the male phase and the appearance of membrane labyrinths at the germinal pore (initiated at the female phase). These two topics provide promising new areas of research both in *Acacia* and in other Mimosoideae.

III. The Stigma

Stigmatic characters appear to have potential for the taxonomist and the student of phylogeny, as has already been indicated by Small & Brookes (1983). Table 1 provides the reader with comparisons of the different types of pistil structures and secretions in each of the three subfamilies. Representative examples of some of these characters can be found in the plates of the next three papers, and we have cross-referenced such illustrations in Table 1 (Owens, Kenrick & Knox, Shivanna & Owens).

Some of the greatest variation in stigma form is found in the Caesalpinioideae, where both WN (wet, nonpapillate) and WP (wet, papillate) forms occur (following the classification of Y. Heslop-Harrison, 1981). The stigmas in Mimosoideae are similar to WN forms in Caesalpinioideae and are apparently fairly uniform. The development of a postpollination exudate in *Acacia* has not been found in members of the other subfamilies. In the Papilionoideae significant morphological and positional variation exists even though the predominant stigma form is WP. In addition, the massing

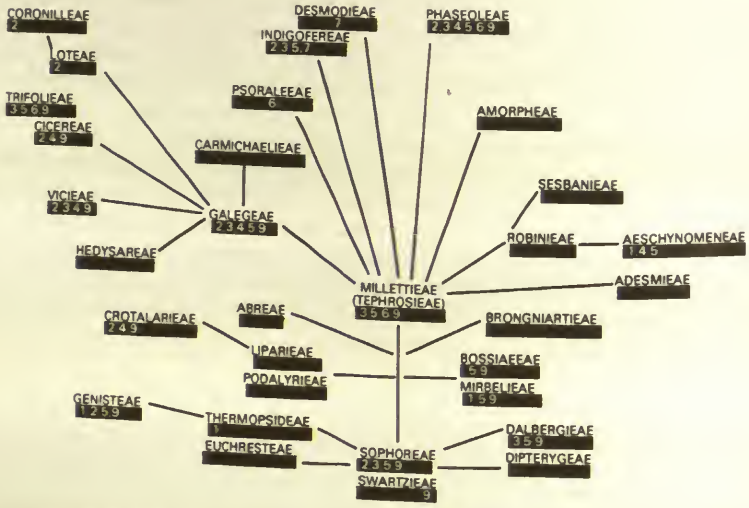

Figure 1. Distribution in the Papilionoideae of entases, types of stigma, presence of hairs on the style, and occurrence of hollow styles. Diagram is redrawn for Polhill (1981, fig. 4) and represents the supposed relationship of the tribes of the Papilionoideae. Key: 1 = DP (dry papillate); 2 = WP (wet papillate); 3 = WN (wet nonpapillate); 4 = hairs present on style; 5 = hairs absent on style; 6 = entasis dominant; 7 = entasis occurs; 8 = entasis absent; 9 = style hollow.

TABLE 1. Summary of presence and absence of stigma and style characters in the three subfamilies of Leguminosae (*Owens; ●Kenrick & Knox; ▲Shivanna & Owens; number = figure in paper).

Stigma	Mimosoideae	Caesalpinioideae	Papilionoideae
Position	Terminal	Terminal	▲1–11 Terminal/Subterminal ▲15
Receptive area	Minute	Minute to very large	Minute to large
Pellicle	–	+/–	+
Cuticle	–	+/(–)	+
Form			
a) WP (wet, papillate)	–	*1–2 +	▲2, 9–11 +/–
b) WN (wet, nonpapillate)			
i) Flat	–	+	+/–
ii) Cup or Crater	●1B +	*8 +	–
iii) Chamber	–	*5–7, 9 +	–
c) DP (Dry, papillate)	–	–	–
Hairs	+/–	*5–8 +/– *1–2	▲4, 6–11 +/– ▲1–3, 5
Secretions			
a) Prepollination			
i) Sparse	–	–	▲1–2, 10–11 +
ii) Copious	●2C +	*10 +	▲3 +
iii) Droplet	+	*3 +	▲4, 6 –
b) Postpollination	●6A +	–	–
Style			
Shape			
a) Cylindrical or tapering	?	+	▲61–62 +
b) Entasis (stylar swelling)	?	–	+/–
c) Post entasis thinning of style	?	–	+/–
Anatomy			
a) Vascular bundle number	1 (Acacia)	>3	>3
b) Transmitting tissue			
i) Solid	+	*13 +	–
Extracellular matrix	+	–	–
ii) Hollow			
Entire ring			
Stylar canal cells	–	*11 +	–
Few localized cells	–	?	–
Cellular occlusion of cavity	–	–	+/–
iii) Lysigenous	–	*12 +	–
iv) i iii ii	–	–	▲18, 21–22, + 31–33, 34–36, 52

–, absent; ?, no data; +, present; (–), insufficient data

of exudate beneath a cuticle (released following cuticle rupture when the flower is tripped) is unique to the subfamily Papilionoideae. The relationship of flower structure, pollinator, and stigma type is still largely unresolved, although an attempt has been made to approach the problem (see Schrire, this volume). Stigmatic chemistry appears uniform throughout the family, but there has been little critical analysis using the more sophisticated separation techniques.

Although Table 1 indicates similarities and differences among the subfamilies, it fails to show which groups are not included in these data and where greater emphasis might be placed in order to aid the resolution of problems relating to phylogeny. Figure 1 demonstrates that the primitive

groups of Papilionoideae, used as an example here, have either not been examined at all or have been given only a cursory examination.

IV. The Style

Stylar anatomy is described for *Acacia* (Kenrick & Knox, this volume), but the main focus of attention in the three papers is the transmitting tissue (Table 1). In the Caesalpinioideae, the transmitting tissue may be hollow, solid, or derived lysigenously, depending on the genus; in the Mimosoideae, transmitting tissue is solid in *Acacia;* in the Papilionoideae, a small portion of the upper style is derived lysigenously, while the larger basal portion is hollow, appearing to extend directly into the stylar canal. Styles in different species of the Caesalpinioideae (Owens, this volume) appear to provide examples of all known forms of stylar transmitting tissue. This appears to contrast with the relative uniformity of those in the Mimosoideae and Papilionoideae. However, the variation in style structure and in the structure of cells lining the canal of species of Papilionoideae certainly merits closer examination. There is considerable justification for broadening this to include the other subfamilies, particularly with regard to pollen tube growth, numbers of pollen tubes, and the site of the self-incompatibility response.

Tissue partitioning in the styles of the Papilionoideae may highlight a potential technical problem. First, in some plants the nature of the walls in the transmitting tissue of the lysigenous and solid types of style make them susceptible to distortion during sectioning of specimens embedded in wax. This may lead to erroneous classification. It may be necessary to use semi-thin sectioning of plastic-embedded material to determine precisely the structure of the style, particularly when it is derived lysigenously from a solid type of style. Second, there has been little work on style development that would aid in the correct interpretation of stylar structure. It may be that all styles are derived from the same form in the Leguminosae and that during evolution the proportion of style that is solid (derived lysigenously) or hollow may have changed. This may have given rise to types such as those in the Papilionoideae, where there is a small area of solid tissue near the stigma, adjacent to an equally small lysigenous area, with a much larger hollow area to the base of the style.

V. Pollen-Stigma Interaction and Pollen Tube Growth

The strategies of the different species with regard to the pollen–stigma interaction vary widely. Copious prepollination exudates are a feature of several WN stigmas of the Caesalpinioideae, while pollen–stigma interaction events cause the production of the postpollination exudate in the mimosoid genus *Acacia*. However, there is no evidence that stigmas in any species can discriminate between incompatible and compatible pollen (Heslop-Harrison & Heslop-Harrison, 1982a), or between pollens of different species or even genera (Kenrick & Knox, 1981, this volume; Owens, this volume). Many Papilionoideae require the intervention of an insect to trip the flower before the stigmatic secretion is released onto the surface and contacts the pollen. In some instances this is achieved without insect visitation, through explosive torsion of the petals (e.g., in species of *Ulex* and *Indigofera*).

In the Papilionoideae, degeneration of the uppermost rank of stigmatic cells has been reported to occur prior to pollination (Heslop-Harrison & Heslop-Harrison, 1983), while it is the emerging pollen tubes that induce progressive necrosis in the stigma and stylar transmitting tissue in *Acacia*. There is no evidence yet from the Caesalpinioideae. Reduction in the volume of transmitting tissue in the style from stigma to ovary in species of the Caesalpinioideae demonstrates a potential upper limit on the number of pollen tubes that may either enter or pass through the entire length of the style. There is little evidence yet of constraints of this type for the other two subfamilies, but morphological data for the Papilionoideae suggests they may indeed exist. Silow (1931), for

example, has shown in *Trifolium* that pollen tubes freely enter the entasis zone but that only a few tubes grow beyond through to the final portion of the style and ovary. Is the entasis a mechanical barrier, a potential growth disorienting zone, or, as suggested by Heslop-Harrison & Heslop-Harrison (1982a), the site of inhibition of self-incompatible pollen tubes? Is the effect of the often considerable proximal narrowing of the style just beyond the entasis a block to massive pollen tube entry into the ovary? Could stylar transmitting tissue structure be effective in reducing the number of pollen tubes traversing the style in those Papilionoideae where only a restricted number of ovules (1–2) are found (e.g., in the Psoraleeae) or where seed set per pod is low? Lord & Kohorn (1986) and Shivanna & Owens (this volume) already suggest such possibilities. What is the significance of the cells that partially occlude the stylar canal near the ovary (Shivanna & Owens, this volume) and of the trichomes in the ovaries of *Acacia* (Kenrick & Knox, this volume)?

Most of the work on pollen tube growth has been accomplished in *Acacia* and the Papilionoideae. The discovery of nucellar inhibition of pollen tubes in incompatible matings in *Acacia* is very significant (Kenrick et al., 1986), and a survey of the Leguminosae is now required to establish whether this is the exception or the rule. Unfortunately, this has been hampered, in our experience, by problems in applying the aniline blue fluorescence technique to species in the other subfamilies and particularly in Caesalpinioideae, where autofluorescence causes pollen tubes to be obscured.

Another significant advance relating to successful fertilization in Papilionoideae is the position of pollen tubes in a stylar canal. Do they fill the stylar canal or do they grow close to the cell walls of a few stylar canal cells? Is this dependent upon the presence of secretion in the canal or the presence of a protein layer alone? Are their synergistic effects related to the number of growing pollen tubes at any particular stage?

VI. The Future

As we have indicated, the three papers that follow stress different emphases not only in the range and choice of material studied but also in the techniques used. It is clear that much data still needs to be gathered and that for the phylogeneticist the results are not exhaustive enough to draw any detailed conclusions at the subfamilial or tribal levels at present. Trends are indicated particularly in relation to ancestral stock of Mimosoideae and Caesalpinioideae. In Table 2 and in the following list, we indicate the many actual and potential constraints that the pistil imposes on pollen germination and tube growth. The model in Table 2 is a predictive one, in that while many of the suggested constraints are not yet supported by good experimental evidence, they are at least based on direct observation.

1. *Ovule number.* An ovule number smaller than the number of pollen tubes arriving in the ovary may enhance male gamete selection and maximize seed set.

2. *Pattern of vascular tissue.* The position of ovules on the ovary wall sutures is related to the pattern of vascular tissue and may or may not determine the order in which ovules are fertilized or whether seed will develop to maturity.

3. *Size of pollen tube transmitting tissue.* The number of pollen tubes able to traverse the ovary is restricted by the size (area and/or volume) of pollen tube transmitting tissue.

4. *Ovary trichomes.* Do these act as a physical barrier to pollen tubes (Kenrick & Knox, this volume)?

5. *Post entasis thinning and length of constriction zone.* Thinning of the style between the base of the entasis and the ovary is a common feature in Papilionoideae, especially in groups whose ovaries set only one or two seeds. Thinning results in a massive reduction of transmitting tissue and hence places a restriction on the number of pollen tubes which will reach the ovary (Silow, 1931). The length of this constriction zone is quite variable in the different tribes and may be a determinant in how long pollen tubes take to reach the ovary. In addition, thinning enhances the flexibility of the pistil. This may be important in chasmogamous flowers that are repeatedly visited by a number

TABLE 2. A generalized model of physical and chemical constraints on pollen germination and tube growth in legume pistils and ovaries.

	Ovary	Style		Stigma
Physical Constraints	Ovule number Pattern of vascular tissue Size of pollen tube transmitting tissue Ovary trichomes	Post entasis thinning and length of constriction zone Cellular occluding hairs	Style structure (solid, hollow, or derived lysigenously) Transition zones Size of transmitting tissue (top, midpoint, and base of style) Entasis	Presence of cuticle Thickness of cuticle and tripping requirement Exposure of stigma, timing and period of receptivity Nonreceptive stigmatic hairs Length of stigmatic papillae Surface area and volume of the stigma Cellular structure
Chemical Constraints	Chemotactic secretions Recognition chemicals of the SI system at the nucellus *(Acacia)*		Quantity and quality of the extra cellular matrix Proteinaceous area receiving and guiding pollen tubes Glycoproteins of the SI system *(Trifolium)*	Pre- and post-pollination exudate Recognition chemicals: intra-, interspecific, and intergeneric

of diverse insects, as it lessens the chance of the pistil being broken, at least before successful fertilization. Finally, is the length of the style correlated with the size of ovary?

6. *Cellular occluding hairs*. Do these hairs impede the passage of pollen tubes? (See Shivanna & Owens, fig. 51, this volume.)

7. *Stylar structure* (solid, hollow, or derived lysigenously). The different types of stylar transmitting zone provide potential barriers to pollen tubes in wide crosses between taxa where the style structure is substantially different. But is the shape of the transmitting tissue limiting in any way?

8. *Transition zones*. Transition zones between tissue and/or fluid provide different growth matrices and hence different physical barriers for the pollen tube to traverse.

9. *Size of transmitting tissue* (top, midpoint, and base of style). A gradual reduction or narrowing of the style transmitting tissue necessarily restricts the number of pollen tubes reaching the ovary (Owens, this volume).

10. *Entasis*. The presence of an entasis might result in a number of functions, for example, disorientation of pollen tubes and mechanical obstruction resulting from toughened tissues. However, the entasis also provides physical strengthening of the androecial column at the point of flexure of the pistil and androecium.

11. *Presence of cuticle*. The cuticle may determine retention and protection of stigmatic secretions and may also act as a barrier to self and/or cross pollen penetration.

12. *Thickness of cuticle and tripping requirement*. The incidence of autogamy and allogamy is directly affected by cuticular thickness (e.g., *Vicia*, Lord & Heslop-Harrison, 1984). Greater force will be required to rupture a thicker cuticle. Cuticle thickness may also provide a barrier to wider crossing if pollen tubes are unable to penetrate the intact cuticle.

13. *Exposure of stigma, timing, and period of receptivity.* In certain legume flowers, the stigma is exposed at maturity. This may affect cell turgor, the amount of stigmatic secretion, and the integrity of the cuticle, which in turn will provide different combinations of barriers to pollen germination and tube growth. Likewise, is the state of hydration of the deposited pollen, which may have originated from exposed or hidden stamens, correlated with stigma type? If receptivity is restricted to a short period during the day, it may decrease the probability of transfer of foreign pollen by restricting effective pollinator visits.

14. *Nonreceptive stigmatic hairs.* Multiple functions are proposed: these hairs may protect the flower from its own pollen; they may aid electrostatic attraction and retention of pollen; they may protect the delicate stigmatic surface until it is receptive; and they may aid cuticular rupture.

15. *Length of stigmatic papillae.* Long, closely packed papillae may act as a sieve against foreign pollen. Alternatively, the presence of very short papillae may mitigate against foreign pollen retention. The type of exine microtexture may be correlated with the type of stigmatic surface.

16. *Surface area and volume of stigma.* Area and volume are correlated with size and number of pollen grains that may adhere, hydrate, and germinate. In pre- and postpollination exudates, is surface tension a significant character in retention of pollen grains?

17. *Cellular structure of the stigma.* Is the internal anatomy of the stigma correlated with surface area and volume? Does cell number affect the amount of secretion and the length of the period of receptivity? Are stigma cells structurally and functionally homogenous?

18. *Chemotactic secretions.* No evidence exists. How is the pollen tube directed to the micropyle? What determines the sequence of ovule fertilization (see Bawa & Buckley, this volume, who show that the pattern of fertilization is not random)? Is the response chemotactic or geotropic?

19. *Recognition chemicals of self-incompatibility system at the nucellus.* This phenomenon has been found only in *Acacia* to date (Kenrick & Knox, this volume). How widespread is this site of inhibition for self-incompatible legumes?

20. *Cellular occluding hairs.* Do they secrete substances (Shivanna & Owens, this volume, fig. 51)?

21. *Quantity and quality of the extracellular matrix.* Are these characters effective in the discrimination of pollen tubes? Can they influence pollen tube growth either positively or negatively?

22. *Proteinaceous area receiving or guiding pollen tubes.* Why is the size and position of the proteinaceous layer different for different taxa? (See Shivanna & Owens, this volume.)

23. *Glycoproteins of the self-incompatibility system.* Glycoproteins are believed to perform roles in the recognition process. Does the presence of glycoproteins indicate self-incompatibility in all cases, and, if so, could it provide a quick bioassay of the self-incompatibility response?

24. *Pre- or postpollination exudate.* What does the exudate contain? The exudates are chemically heterogenous, but are some of the chemicals genus or species specific?

25. *Recognition chemicals.* Can stigmas of any of the legumes discriminate between pollens of different species or genera?

Many unanswered questions have been raised. We would hope that future students will consider these different aspects in their studies, and we encourage them to take on a broader range of legumes, from both primitive and advanced groups.

References

Brewbaker, J. L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the Angiosperms. Amer. J. Bot. 54: 1069–1083.

Ferguson, I. K. 1987. A preliminary survey of the pollen exine stratification in the Caesalpinioideae. Pp. 355–385 *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

_____ & J. J. Skvarla. 1981. The pollen morphology of the subfamily Papilionoideae (Leguminosae). Pp. 859–896 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Graham, A. & G. Barker. 1981. Palynology and tribal classification in the Caesalpinioideae. Pp. 801–834 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Heslop-Harrison, J. & Y. Heslop-Harrison. 1982a. Pollen–stigma interaction in the Leguminosae: constituents of the stylar fluid and stigma secretion of *Trifolium pratense* L. Ann. Bot. 49: 729–735.

_____ & _____. 1982b. Pollen–stigma interaction in the Leguminosae: the secretory system of the style in *Trifolium pratense* L. Ann. Bot. 50: 635–645.

_____ & _____. 1983. Pollen–stigma interaction in the Leguminosae: the organization of the stigma in *Trifolium pratense* L. Ann. Bot. 51: 571–583.

_____ & _____. 1984. Stigma organization and the control of fertilization in *Phaseolus*. Pp. 88–98 *in* R. Reimann-Philipp (editor), Proceedings of the Eucarpia meeting on *Phaseolus* bean breeding, Hamburg, July 1983.

Heslop-Harrison, Y. 1981. Stigma characteristics and angiosperm taxonomy. Nordic J. Bot. 1: 401–420.

Kenrick, J. & R. B. Knox. 1981. Post-pollination exudate from stigmas of Acacia (Mimosaceae). Ann. Bot. (London) 48: 103–106.

_____, V. Kaul & E. G. Williams. 1986. Self incompatibility in *Acacia retinodes:* site of pollen tube arrest is the nucellus. Planta 169: 245–250.

Lord, E. M. & Y. Heslop-Harrison. 1984. Pollen–stigma interaction in the Leguminosae: stigma organization and the breeding system in *Vicia faba* L. Ann. Bot. 54: 827–836.

_____ & L. U. Kohorn. 1986. Gynoecial development, pollination, and the path of pollen tube growth in the Tepary bean, *Phaseolus acutifolius*. Amer. J. Bot. 73: 70–78.

_____ & B. D. Webster. 1979. The stigmatic exudate of *Phaseolus vulgaris* L. Bot. Gaz. (Crawfordsville) 104: 266–271.

Polhill, R. M. 1981. Papilionoideae. Pp. 191–208 in R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Silow, R. A. 1931. A preliminary report on pollen-tube growth in red clover (*Trifolium pratense* L.). Welsh Pl. Breed. Stn. Bull. Series H, 12: 228–233.

Small, E. & B. S. Brookes. 1983. The systematic value of stigma morphology in the legume tribe Trifolieae with particular reference to *Medicago*. Canad. J. Bot. 61: 2388–2404.

STIGMA, STYLE, POLLEN, AND THE POLLEN–STIGMA INTERACTION IN CAESALPINIOIDEAE

Simon J. Owens[1]

ABSTRACT

Stigmas in species of more than 40 genera have been examined, and two types are recognized: WP (capitate, wet, papillate) and WN (crateriform, wet, nonpapillate). There is considerable morphological variation within each stigmatic type. WP stigmas are found in species of all tribes in the Caesalpinioideae: WN stigmas are only found in the Caesalpinieae and Cassieae. The stigmatic secretion is copious when the stigma is receptive and is histochemically similar in both forms. The stylar transmitting zone is variable in structure. The majority of species have either stylar canals that are derived lysigenously or a solid transmitting tissue. Three species have a hollow canal. Transmitting tissue may diminish in size from stigma to style base, imposing a potential restriction on the numbers of pollen tubes traversing the style. Studies of pollen–stigma interaction have been limited by technique and species availability. The speed to pollen germination is consistent with that of other species with wet stigmas. WN stigmas do not discriminate between pollens.

I. INTRODUCTION

There is little published data describing pollen (except pollen morphology) and pistil in the Caesalpinioideae, and only Owens (1985) has reported data on the pollen–stigma interaction. This paper is a summary of results, many of which have been obtained at Kew; more detailed analyses will follow in later publications.

The aims of this paper are threefold: 1) to indicate the variability in the morphology and anatomy of the stigma and style in different genera and species, and in the nature of the stigmatic secretion; 2) to demonstrate the potential use of such characters in studies of systematics and evolution; and 3) to suggest, where possible, relationships between structure and function.

II. THE STIGMA

STIGMA SURFACE MORPHOLOGY

Stigmatic surfaces vary widely in size and in shape, from capitate (Fig. 1) and bilobed (Fig. 2) to crateriform (cup- or funnel-shaped; Fig. 3). All stigmas are of the wet type and can be categorized using the modified classification of Heslop-Harrison (1981). This confirms the groupings for Caesalpinioideae suggested by Owens (1985).

(a) WP-receptive cells papillate; secretion moderate to slight, flooding interstices. Papillae may be variable in length and shape (Figs. 1, 2, 4).

(b) WN-receptive cells nonpapillate, secretion usually copious. The surface often appears smooth, although in most instances it is obscured wholly or partially by fluid (Fig. 3). In most species of *Cassia*, *Chamaecrista*, and *Senna*, stigmatic surfaces cannot be observed directly in receptive stigmas (see Stigma Anatomy below). There is considerable morphological variability in the size and form of the WN stigma, and this is particularly apparent in the genus *Senna* (Figs.

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K.

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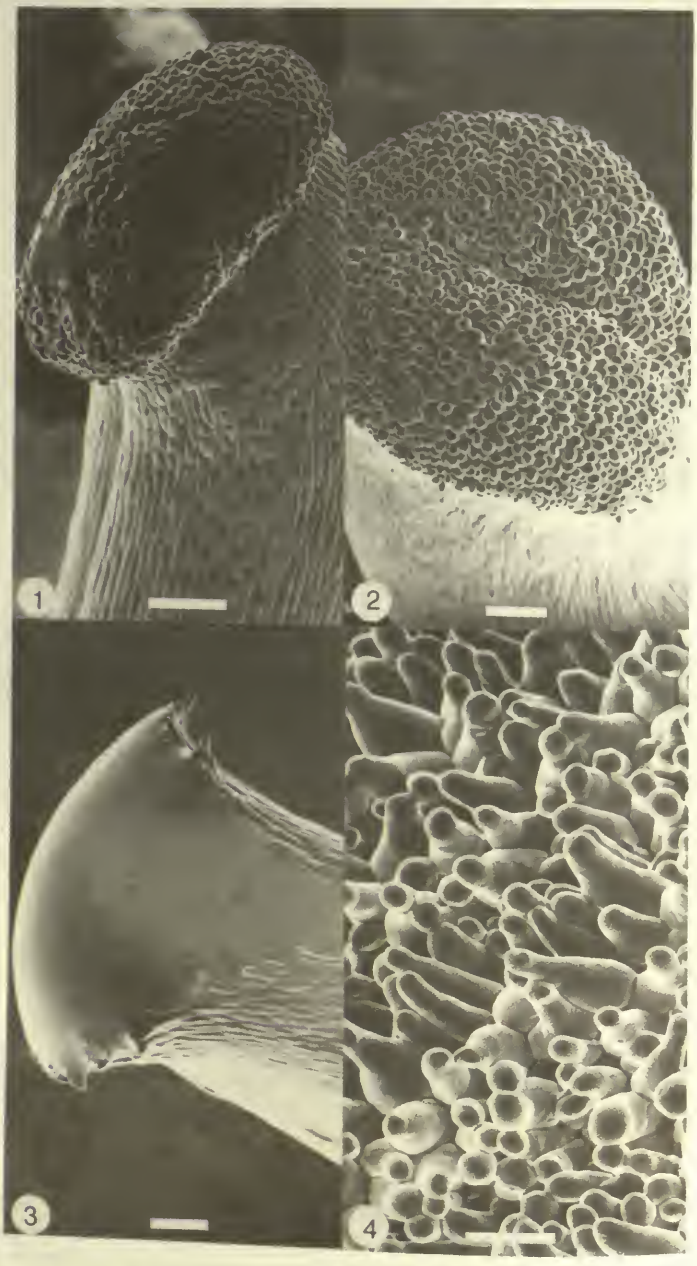
MONOGR. SYST. BOT. MISSOURI BOT. GARD. 29:113–126. 1989.



FIGURES 1–4. Scanning electron micrographs of stigmas removed directly from flowers or flower buds, without treatment or coating. —1, 2. Stigmatic surfaces (WP) of two pistils of *Cercis siliquastrum*. Papillae are short and may become partially or wholly inundated by stigmatic secretion (Fig. 1). The stigma in Figure 2 is clearly bilobed. —3. The crateriform stigma of *Caesalpinia pulcherrima;* stigmatic surface obscured by a secretion droplet. —4. Part of a stigma of *Bauhinia tomentosa* showing the stigmatic papillae. Scale bars = 100 μm.

6–8). In this genus, the porate entrance to the stigmatic chamber shows variation in overall shape and size, and in the number, size, and shape of the hairs which form the entrance fringe.

STIGMA ANATOMY

Sections of WP stigmas reveal the bipartite structure of the solid cellular mass. The surface is delimited by the stigma papillae, a unicellular monolayer. Beneath the papillae is a multilayered cellular zone comprising closely associated elongate cells. The majority of stigmatic secretion appears to be generated in these cells and is transported to an extracellular site, initially the intercellular spaces. This secretory zone connects directly to the stylar transmitting zone and, in the case of solid styles, it may be difficult to determine where the stigmatic secretory zone ends and the transmitting zone begins. There are structural and functional similarities between the stigmatic secretion zone of Caesalpinioideae and those in Papilionoideae (Heslop-Harrison & Heslop-Harrison, 1982; Lord & Kohorn, 1986) and species in other families (e.g., Solanaceae; Dumas et al., 1978).

Sections of WN types show that the stigma structure comprises a depression of variable depth positioned at the apex of the style, which is lined with a multilayered secretory zone of frequently loosely fitting cells. Detached cells are often found free-floating in the stigmatic secretion.

In the tribe Cassiinae, stigmas can be subdivided further into crateriform (Fig. 8) and chambered (Figs. 5, 7, 9). The chambered form is the more common one, found in all the genera (Tables 1, 2); the crateriform type (Fig. 8) has not been found in our studies in the genus *Cassia* (Owens & Lewis, in prep.).

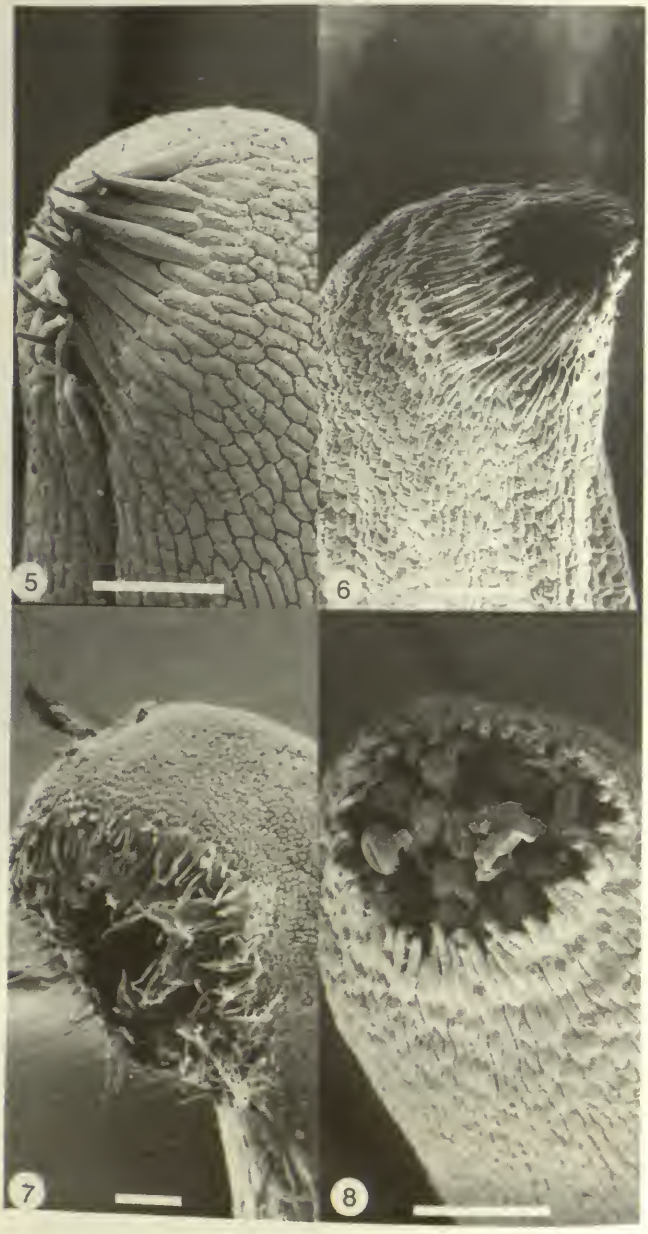
HISTOCHEMISTRY OF STIGMATIC SECRETIONS

Stigmatic secretions are heterogeneous, and proteins (some with esterase activity), lipids, and carbohydrates have been identified using standard histochemical tests (see Heslop-Harrison, 1979). Secretions do not appear to differ in WP and WN stigmas and are apparently little different from those found in many other species, including *Acacia* (Kenrick & Knox, 1981), *Phaseolus,* and *Trifolium* (Heslop-Harrison & Heslop-Harrison, 1983, 1984; Lord & Kohorn, 1986). The secretion is almost certainly hydrophilic and may also contain phenols (Knox, 1984).

The secretion components are partitioned on the stigma surface, and this is clearly indicated in the transmission electron micrograph (Fig. 10). The electron opaque matrix is filled with "vacuoles" of various sizes and shapes. These "vacuoles" may or may not be filled with electron opaque material of granular or fibrillar nature. Differences in the quantity or compartmentalization of components on stigmas of different genera or species cannot be identified by the methods employed to date.

TAXONOMIC AND EVOLUTIONARY RELATIONSHIPS

The distribution of the various stigmatic forms within and between tribes is shown in Table 1. All tribes are found to have genera with the WP type, while only the tribes Caesalpinieae and Cassieae have both the WP and WN type. Table 2 shows the data in more detail for the latter tribes. In the Caesalpinieae, the recognized groups are found to possess genera with either the WP or the WN stigma form. The exception is the Peltophorum group, where *Jacqueshuberia* and *Peltophorum* are genera in the group possessing a WP stigma, and *Delonix* and *Moldenhauera* a WN stigma. In the



FIGURES 5–8.—5. Reflexed stigmatic pore of *Chamaecrista* sp.; porate entrance to the stigmatic surface obscured by the fringe of pore hairs. —6. Open stigmatic pore of *Senna pentandra;* a single layer of hairs delimits the pore. —7. Stigmatic pore of *Senna sophera* showing a multilayered fringe of fine hairs of variable length. —8. Crateriform stigma of *Senna* sp., positioned at the apex of the style and fringed by a single layer of short hairs; pollen grains and pollen tubes are present in the crater. Stigmas fixed, critically point-dried, and coated before photography and observation in Figures 5, 7, 8; Figure 6 without treatment or coating. Scale bars = 100 μm.

TABLE 1. The distribution of stigmatic forms within and between the tribes of the Caesalpinioideae. WP = wet, papillate; WN = wet, nonpapillate; + = form present.

Tribe	Number of groups or subtribes examined/total in group or subtribe	Number of genera and species ( ) examined	WP	WN	
				Crater	Chamber
Caesalpinieae	6/8	16 (22)	+	+	
Cassieae	3/5	6 (35)	+	+	+
Cercideae	2/2	3 (9)	+		
Detarieae	5/6	13 (16)	+		
Amherstieae	4/4	9 (9)	+		

TABLE 2. The distribution of stigmatic forms within and between the groups and subtribes of the tribes Caesalpinieae and Cassieae. WP = wet, papillate; WN = wet, nonpapillate; + = form present.

Tribe/group or subtribe	WP	WN	
		Crater	Chamber
Caesalpinieae			
Gleditsia group	+		
Acrocarpus group	No data		
Sclerolobium group	+		
Peltophorum group	+	+	
Caesalpinia group		+	
Poeppigia group	+		
Pterogyne group	No data		
Dimorphandra group		+	
Cassieae			
Ceratoniinae	No data		
Dialiinae	+		
Duparquetiinae	+		
Cassiinae		+	+
Labicheinae	No data		

Cassiinae, the genera *Chamaecrista* and *Senna* have both chambered and crateriform morphs (cf. Figs. 8, 9).

It is interesting to note that the stigmatic surfaces of species in the Mimosoideae have porate, cupular, or funnel-shaped stigmas while species in the Papilionoideae have solid, papillate stigmas. Structurally and chemically these two stigmatic forms are similar to some of the forms in the Caesalpinioideae. The occurrence of the WP stigmatic form in the Sclerolobium group and the WN form in the Dimorphandra group (Table 2, Caesalpinieae) support the contention that the Papilionoideae arose from the former and the Mimosoideae from the latter (Polhill et al., 1981).

III. The Style

MORPHOLOGY

The style varies considerably in external morphology, particularly in length. For example, it is long and rigid in *Baikiaea insignis* (60 mm), long and flexuous in *Caesalpinia gilliesii* (85 mm), short and rigid in *Cercis siliquastrum* (8 mm), and very short in some *Bauhinia* species, *Griffonia*,

and *Senna*. There have been no attempts to correlate style length and style anatomy, stigma depth, or pollen grain size (Cruden & Lyon, 1985).

ANATOMY OF THE STYLAR TRANSMITTING ZONE

The transmitting tissue provides yet a further variable parameter (Table 3). All three types of stylar transmitting zone, as originally described by Hanf (1933), have been found in open flowers of species of the Caesalpinioideae:

(a) A hollow canal is present, bordered by one or more layers of cells (Fig. 11). These cells do not appear structurally similar, when viewed with the light microscope, to those of monocotyledonous genera such as *Lilium* (Liliaceae) (Dickinson et al., 1982) and *Tradescantia* (Commelinaceae) (Owens et al., 1984). Little further data is available, although the zone appears structurally similar to that found in genera of the Papilionoideae such as *Cajanus, Crotolaria,* and *Vigna* (Ghosh & Shivanna, 1982) and *Phaseolus* (Lord & Kohorn, 1986). It has only been identified in three Caesalpinioid species, all of which have WP stigmas (Table 3).

(b) A more or less hollow, fluid, and cell-filled (lysigenous) canal is present (Fig. 12). Light microscope studies of styles from young buds indicate that the zone was a solid, cellular tissue, but electron microscopic studies of *Caesalpinia* and *Senna* clearly show a canal (Owens, unpublished). The middle lamella of the cells of this tissue breaks down and the cells become partially or wholly free from each other. Concomitant with this breakdown is an increase in fluid in the space left by the disorganizing mass. Cell death is apparent in some but not all free-floating cells. Species with lysigenous stylar canals are found to have either WP or WN stigmas; WN stigmas are, however, only found to have lysigenous canals (Table 3).

(c) A solid cellular transmitting tract is found (Fig. 13), which is cytologically similar to that in species such as *Petunia* (Solanaceae) (Herrero & Dickinson, 1979). All species have a WP stigma.

TAXONOMIC RELATIONSHIPS

There is no apparent relationship of stylar transmitting tissue type to taxonomic grouping at the tribal level.

STRUCTURE/FUNCTION RELATIONSHIPS

The preliminary results (Owens & McGrath, unpublished) of stylar reconstruction using microcomputer-aided serial reconstruction techniques suggest that in some species reduction in the size of the stylar transmitting zone from stigma to style base could restrict the number of pollen tubes entering the ovary. The change has been found to be dramatic in *Cercis siliquastrum* (solid stylar transmitting zone) and *Delonix regia* (lysigenous transmitting zone). In *Brownea* × *crawfordii* the pathway for pollen tubes in the style is uniformly narrow from stigma to ovary.

Recently, Lord & Kohorn (1986) have shown that the secretory transmitting tract in the hollow style of *Phaseolus acutifolius* is restricted to the ventral side of the stylar canal and suggested that this causes a restriction in the number of pollen tubes that can be accommodated in the style because

TABLE 3. Genera with differing transmitting tissue type. Stigmatic morph and tribal classification are included for comparison. WP = wet, papillate; WN = wet, nonpapillate.

Transmitting zone type/genus	Stigmatic morph	Tribal classification
Stylar canal		
Duparquetia	WP	Cassieae
Baikiaea	WP	Detarieae
Intsia	WP	Detarieae
Lysigenous zone		
Campsiandra	WN	Caesalpinieae
Caesalpinia	WN	Caesalpinieae
Pterolobium	WN	Caesalpinieae
Bauhinia	WP	Cercideae
Senna	WN	Cassieae
Griffonia	WP	Cercideae
Brodriguesia	WP	Detarieae
Brownea	WP	Detarieae
Daniella	WP	Detarieae
Saraca	WP	Detarieae
Brachystegia	WP	Amherstieae
Solid tissue		
Gleditsia	WP	Caesalpinieae
Jacqueshuberia	WP	Caesalpinieae
Poeppigia	WP	Caesalpinieae
Cercis	WP	Cercideae

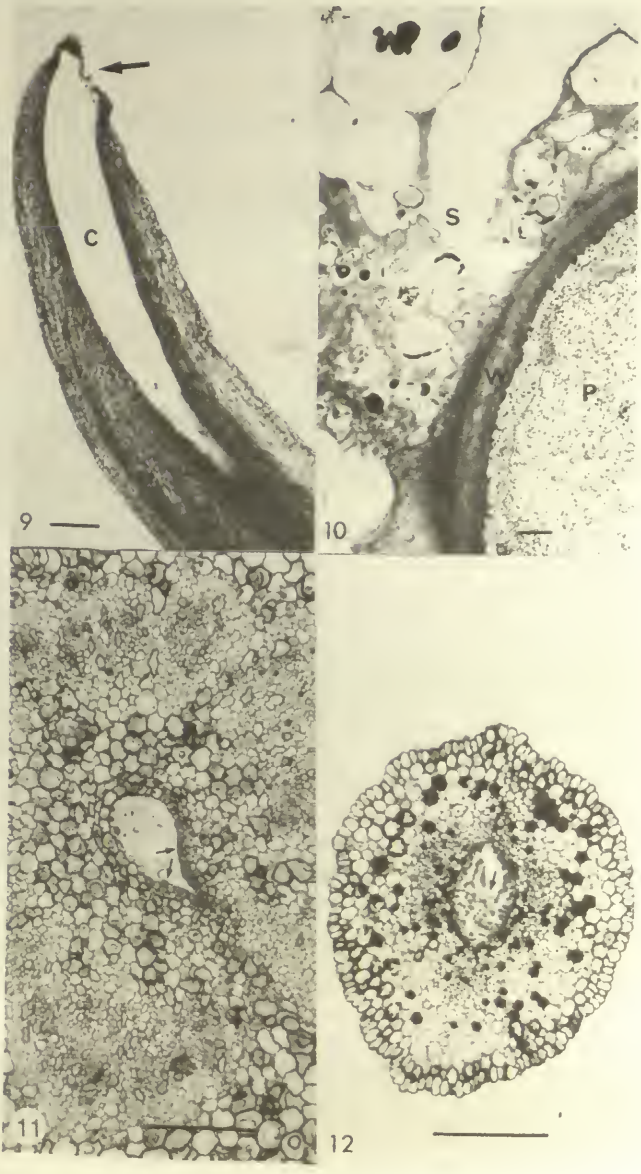
all tubes must be closely associated with the secretory area. Their results show that the numbers of pollen tubes are indeed reduced from those on the stigma surface to those that enter the canal zone. No data is available yet for Caesalpinioideae, but clearly the hollow style types require further work regarding such a feature, since it proves an additional constraint to pollen tube growth. The canal cells shown in Figure 11 appear to show a degree of differentiation. Cells on the ventral side of the canal are smaller, more densely cytoplasmic, and less vacuolate than those on the dorsal side.

IV. The Pollen Grain

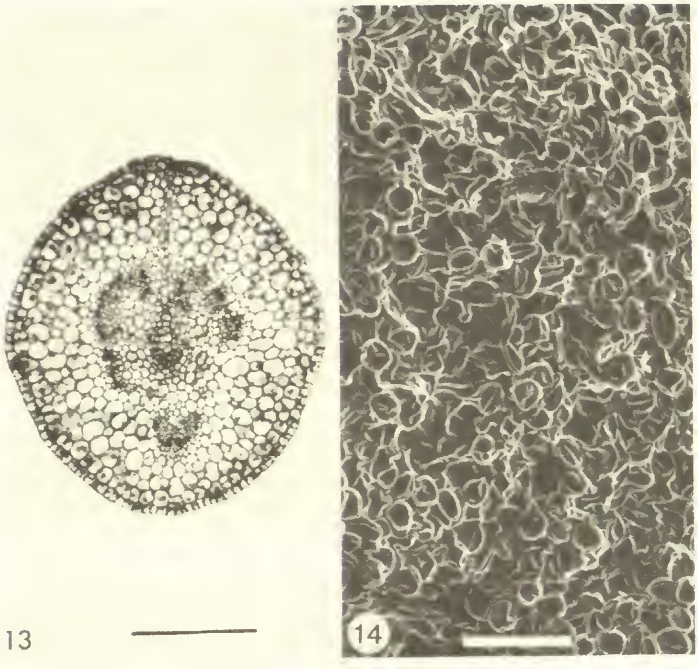
CYTOLOGY

Anatomy. Pollen grains are single units in the majority of species but are released as tetrads in *Afzelia* and *Diptychandra* (Graham & Barker, 1981). In these species, occurrence of tetrads is not associated with a situation like the polyad/stigmatic cup/ovule relationship of the Mimosoideae (Knox & Kenrick, 1983). Stigmas of *Afzelia* that have been examined are of the WP type. *Diptychandra* species have not been available for investigation, but species of the genera *Sclerolobium* and *Tachigali*, placed in the same taxonomic grouping (Caesalpinieae : Sclerolobium group), also have stigmas of the WP type. In addition, the Sclerolobium group is believed to have strong evolutionary links to the Papilionoideae (Polhill et al., 1981).

Starch/starchless. Representatives of the family were described as starchless (Baker & Baker, 1979). Unpublished Kew data indicate that the large pollen grains of *Bauhinia tomentosa* and *B. pentandra* and the pollen of *Cercis siliquastrum* may possess starch and lipid, and the pollen of *Caesalpinia gillesii* appears starchless.



FIGURES 9–12. Pistil material: fixed, dehydrated and embedded in paraplast using conventional techniques (Figs. 9, 11, 12); Figure 10 prepared by conventional techniques used in transmission electron microscopy. —9. Section (LS) through the stigmatic chamber of *Senna floribunda*. Arrow shows stigmatic pore and hairs; C = stigmatic chamber. —10. Transmission electron micrograph of stigmatic surface of *Cercis siliquastrum* showing heterogeneous stigmatic secretion (S). P = stigma papillar cell; W = papillar cell wall. —11. Section through style of *Baikiaea insignis*. The hollow stylar canal is bordered by a layer of canal cells. Arrows indicate smaller, denser canal cells. —12. Section through a style of *Brownea* × *crawfordii*. Lysigenous stylar transmitting zone is clearly visible and contains free-floating cells and cell debris (arrow). Scale bars = 100 μm for Figures 9, 11, 12; 1 μm for Figure 10.



FIGURES 13, 14.—13. Section through a style of *Cercis siliquastrum*. The solid area of cells comprising the transmitting zone is almost entirely surrounded by vascular tissue. —14. Part of a pollinated stigma of *Gleditsia japonica* showing general stigma papilla collapse. Not all pollen grains have hydrated or germinated (arrows indicate two germinated grains). Scale bars = 100 μm.

Number of nuclei. Ten of 135 genera have been examined, with representatives from all tribes in the subfamily, and all are binucleate (Brewbaker, 1967).

THE POLLEN WALL

Exine morphology relationship to the stigma. No character in the morphology of the exine has yet been linked to a particular stigma type. Exine morphology can, in some species, be linked to pollinator (Graham & Barker, 1981; Ferguson & Pearce, 1986).

The results of the interspecific crosses herein and previously (Owens, 1985) indicate that any divergence in pollen structure does not affect the ability of any one type of pollen to germinate on a foreign stigma. It would be surprising, therefore, to find specific correlations between pollen structure and stigma type, particularly in the species which were available for the crossing experiment. However, a number of genera do indicate interesting possibilities. In *Bauhinia* there are at least two types of pollen grains (Ferguson, pers. comm.), and there appear to be two types of stigma (Owens, unpublished). In *Berlinia* there are two types of pollen grains and there are stigmas which possess skittle-shaped papilla cells and stigmas that do not (Owens, unpublished). Viscin threads have been positively identified in pollen of *Jacqueshuberia* (Graham & Barker, 1981; Graham et al., 1980). Their occurrence elsewhere is controversial (see Pollenkitt below; Cruden & Jensen, 1979; Hesse, 1984a, b; Patel et al., 1985).

Pollenkitt. Recently Hesse (1984a, b) has found threads connecting unacetolyzed pollen of *Cae-*

TABLE 4. The response of self-pollen on the stigma. WN = wet, nonpapillate; WP = wet, papillate; + = pollen tubes observed; − = pollen tubes not observed; nt = not tested.

Self-pollination stigma type	Pollen germination	Time in minutes to produce first observable tubes
WN		
Caesalpinia gillesii	+	30
C. pulcherrima	+	43
Senna floribunda	+	20
S. nemophila	−	>120
S. obtusa	+	nt
S. pentandra	−	nt
WP		
Baikiaea insignis	+	nt
Bauhinia candicans	+	nt
B. pentandra	+	180
B. tomentosa	+	60
Brownea × *crawfordii*	+	nt
B. coccinea × *latifolia*	+	nt
Cercis chinensis	+	60
C. occidentalis	+	nt
C. siliquastrum	+	55
Gleditsia caspica	+	nt
G. japonica	+	nt

salpinia pulcherrima and *Delonix alata*. Hesse (1984a, b) suggests that these threads are not viscin threads but are filiform tapetal derivatives (ropy pollenkitt) which function to hold pollen grains together in numbers greater than one during transport and transfer to the stigma.

V. The Pollen–Stigma Interaction

Intraspecific pollinations

Pollen germination after manual controlled self-pollination has been successfully undertaken for most species (Table 4). The time taken for pollen to germinate on the stigma varies according to species between 30 and 60 minutes, with *Bauhinia pentandra* being an exception at 180 minutes. Germination is more rapid in those species with WN stigmas than with WP (Table 4; Owens, 1985). Interplant crosses of the same species are limited by the lack of replicates at Kew, but very recent unpublished work at Kew suggests that there is little or no difference between the speed of germination in selfs and interplant crosses. The time taken to germination does fall into the moderate to slow range that would be expected in stigmas of this type (Knox, 1984; Heslop-Harrison, 1985).

Attempts at pollen germination in two species, *Senna nemophila* and *S. pentandra*, have failed almost certainly for different reasons, neither of which appears to involve pollen sterility. In *S. pentandra* stigmas have rarely, if ever, produced a secretion droplet under Kew conditions, a feature necessary for pollen germination in *S. floribunda* (Owens, 1985) and almost certainly related to stigma receptivity in this species. In *S. nemophila* secretion droplets are always present but pollen germination is still not observed. Apomixis has been recorded for the group of which *S. nemophila* is a member and may have a bearing on the lack of pollen germination (Randell, 1970). There is no evidence for a postpollination secretion response in the WN stigma of Caesalpinioideae as is found in *Acacia* (Kenrick & Knox, 1981).

TABLE 5. The response of pollen grains on stigmas in interspecific and intergeneric combinations (modified, with additions from Owens, 1985). WN = wet, nonpapillate; WP = wet, papillate; + = pollen germination; − = no germination; (+) = <1 pollen grain germinated/stigma.

Cross combination	Response	Minimum time to pollen germination in minutes
WN × WN		
Senna obtusa × *S. floribunda*	+	35
S. obtusa × *Caesalpinia pulcherrima*	+	45
S. floribunda × *C. pulcherrima*	+	45
S. floribunda × *C. gilliesii*	+	45
C. gilliesii × *S. floribunda*	+	30
WN × WP		
S. floribunda × *Bauhinia tomentosa*	+	30
C. pulcherrima × *B. pentandra*	+	190
C. pulcherrima × *Cercis siliquastrum*	+	60
WP × WN		
Cercis siliquastrum × *C. pulcherrima*	(+)	1,700
WP × WP		
C. siliquastrum × *B. pentandra*	−	>2,940

INTERSPECIFIC AND INTERGENERIC POLLINATIONS

The results of Owens (1985) show that the germination of pollen from different species or even genera on the stigmas of *Senna* and *Caesalpinia* are as rapid or almost as rapid as for self pollen. Since this publication, a greater number of replicates has been selfed and a wider range of species are now included; these data are presented in Table 5. Foreign pollen is easily applied to the stigmatic droplet on the WN stigma, sinks into the secretion, and is retained. Adherence to the cells of the stigma surface does not take place and pollen can be lifted from the droplet at any time before stylar penetration of the pollen tube. The result confirms that the stigmatic surfaces of WN species have no apparent ability to discriminate between pollens. In this respect they are similar to the solid papillate stigma of *Citrullus lanatus* (Cucurbitaceae) (Sedgely & Blesing, 1982), where foreign pollen will produce the same stigmatic responses as does self- or intraspecific cross pollen. In this case, too, pollen germinates.

The germination response is different where solid papillate stigmas have been the recipients of foreign pollen, and in this they differ considerably from *Citrullus lanatus* (Sedgely & Blesing, 1982). In the experimental pollinations carried out using the solid stigma of *Cercis siliquastrum* it has proven difficult to place the very large pollen grains of *Bauhinia pentandra* on the stigma surface, and it was only slightly easier with the smaller *Caesalpinia pulcherrima* pollen grains. The germination response was very slow and sporadic in the latter (ca. 28 hrs.), and pollen adherence and germination was not observed in the former even after 49 hours. Characteristics such as pollinator specificity, geographical separation, and/or a difference in flowering time can be sufficient to prevent such contamination from occurring.

VI. POLLEN TUBE GROWTH

PATHWAY OF THE POLLEN TUBE IN THE STYLE

There is no data on pollen tube growth available for any species in the Caesalpinioideae. If one extrapolates from data in Papilionoideae (Lord & Kohorn, 1986) and Mimosoideae (Kenrick & Knox, 1985), then the pathway of the pollen tube is the stylar transmitting zone as identified above

(Section III). The work of Lord & Kohorn (1986) indicates, however, that the whole area of transmitting tissue may not provide a pathway for pollen tubes.

A major difficulty yet to be overcome in the Caesalpinioideae is the inability of any method using the fluorochrome aniline blue to permit observation of pollen tubes in the style. Autofluorescence and perhaps induced fluorescence in the style obstructs observation of pollen tubes; tubes and plugs may be fluorescing faintly as has been found in *Acacia retinodes* (Kenrick & Knox, 1985). No data has been obtained concerning the number of pollen tubes traversing the style.

VIII. Self-Incompatibility

The presence of gametophytic self-incompatibility in Caesalpinioideae has been suggested by Arroyo (1981) based on three lines of indirect evidence. Binucleate pollen and wet stigmas have been shown to be correlated with gametophytic self-incompatibility (Brewbaker, 1967; Heslop-Harrison & Shivanna, 1977, respectively), and both are present in species of Caesalpinioideae (also confirmed in this paper). Gametophytic self-incompatibility has been indicated for several species in Papilionoideae and further confirmed by genetic analysis in at least three (Bubar, 1958; Duvick, 1966). It has also been proposed for *A. retinodes* (Knox & Kenrick, 1983; Kenrick & Knox, 1985). The contention then seems reasonable given the present data.

The reported presence of self-incompatibility in individual species should now, however, be reassessed. Brewbaker (1984) has already indicated that most reports on self-incompatibility are preliminary reports and lack critical data. In addition, there is evidence that species such as *Caesalpinia eriostachys* (Bawa & Webb, 1984) possess high levels of flower, fruit, and even seed abortion, and that *Cassia* species, as reported in this paper, may not develop flowers with receptive stigmas.

VIII. Future Studies

There are numerous characters of the subfamily Caesalpinioideae that need further examination. Self-incompatibility is yet to be fully confirmed in any species, most reports being preliminary observations (Brewbaker, 1984). Relationships of pollinator and pollen to stigma or style are only now being investigated.

The different stigmatic surfaces in the Mimosoideae and Papilionoideae, both of which are found represented in the Caesalpinioideae, support the contention that the Caesalpinioideae are the primitive group. The evolution of the two stigma forms is more difficult to see and requires more data. One hypothesis to be tested, however, is that the WN type is derived from the WP type. Two items of the data in this paper indicate this. First, the WN stigma surface appears to lack an epidermis. It has been lost from a once WP type stigma during evolution, and the stigmatic secretory zone is now exposed. Second, the ability to discriminate between foreign pollens is a feature of the WP stigma. This ability to discriminate may reside in the papillate epidermis and, hence, has been lost in the WN types.

References

Arroyo, M.T. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Baker, H. G. & I. Baker. 1979. Starch in angiosperm pollen grains and its evolutionary significance. Amer. J. Bot. 66: 591–600.

Bawa, K. S. & C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. Amer. J. Bot. 71: 736–751.

Brewbaker, J. L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Amer. J. Bot. 54: 1069–1083.

_____. 1984. Nitrogen-fixing tree breeding. Pp. 53–60 *in* E. G. Williams & R. B. Knox (editors), Pollination '84. School of Botany, Univ. of Melbourne.

Bubar, J. S. 1958. An association between variability in ovule development within ovaries and self-incompatibility in *Lotus* (Leguminosae). Canad. J. Bot. 36: 65–72.

Cruden, R. W. & K. G. Jensen. 1979. Viscin threads, pollen efficiency and low pollen-ovule ratios. Amer. J. Bot. 68: 875–879.

_____ & D. L. Lyon. 1985. Correlations among stigma depth, style length, and pollen grain size: Do they reflect function or phylogeny? Bot. Gaz. (Crawfordsville) 146: 143–149.

Dickinson, H. G., J. Moriarty & J. Lawson. 1982. Pollen–pistil interaction in *Lilium longiflorum:* the role of the pistil in controlling pollen tube growth following cross- and self-pollination. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 215: 45–62.

Dumas, C., M. Rougier, P. Zandonella, F. Ciampolini & E. Pacini. 1978. The secretory stigma in *Lycopersicum peruvianum* Mill.: ontogenesis and glandular activity. Protoplasma 96: 173–187.

Duvick, D. N. 1966. Influence of morphology and sterility in breeding methodology. Pp. 85–138 *in* K. J. Frey (editor), Plant Breeding Symposium. Iowa State Univ., Ames, Iowa.

Ferguson, I. K. & K. J. Pearce. 1986. Observations on the pollen morphology of the genus *Bauhinia* L. (Leguminosae: Caesalpinioideae) in the Neotropics. Pp. 283–296 *in* S. Blackmore & I. K. Ferguson (editors), Pollen and Spores: Form and Function. Academic Press, London & New York.

Ghosh, S. & K. R. Shivanna. 1982. Anatomical and cytochemical studies on the stigma and style in some legumes. Bot. Gaz. (Crawfordsville) 143: 311–318.

Graham, A. & G. Barker. 1981. Palynology and tribal classification in the Caesalpinioideae. Pp. 801–834 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____, _____ & M. F. Da Silva. 1980. Unique pollen types in the Caesalpinioideae. Grana 19: 79–84.

Hanf, M. 1935. Vergleichende und Enywicklungsgeschichtliche Untersuchungen uber Morphologie und Anatomie der Griffel und Griffelaste. Beih. Bot. Centralbl. 54: 99–141.

Herrero, M. & H. G. Dickinson. 1979. Pollen–pistil incompatibility in *Petunia hybrida:* changes in the pistil following compatible and incompatible intraspecific crosses. J. Cell Sci. 36: 1–18.

Heslop-Harrison, J. 1979. Aspects of the structure, cytochemistry and germination of the pollen of rye (*Secale cereale* L.). Ann. Bot. (London) 44, Suppl. 1: 1–47.

_____. 1985. Surfaces and secretions in the pollen–stigma interaction: a brief review. Pp. 287–300 *in* K. Roberts, A. W. B. Johnston, C. W. Lloyd, P. Shaw & H. W. Woolhouse (editors), The Cell Surface in Plant Growth and Development. J. Cell Sci. Suppl. 2.

_____ & Y. Heslop-Harrison. 1982. Pollen–stigma interaction in the Leguminosae: constituents of the stylar fluid and stigma secretion in *Trifolium pratense* L. Ann. Bot. (London) 49: 729–735.

_____ & _____. 1983. Pollen–stigma interaction in the Leguminosae: the organization of the stigma in *Trifolium pratense* L. Ann. Bot. (London) 51: 571–583.

_____ & _____. 1984. Stigma organization and the control of fertilization in *Phaseolus*. Pp. 88–96 *in* R. Reimann-Philipp (editor), Proc. Eucarpia Meeting on Phaseolus Bean Breeding. Hamburg, July 1983.

Heslop-Harrison, Y. 1981. Stigma characteristics and angiosperm taxonomy. Nordic J. Bot. 1: 401–420.

_____ & K. R. Shivanna. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. (London) 41: 1233–1258.

Hesse, M. 1984a. Form and function of *Delonix* pollen surface. Mikroscopie 41: 70–72.

_____. 1984b. An architecture model for viscin threads. Grana 23: 69–75.

Kenrick, J. & R. B. Knox. 1981. Structure and histochemistry of the stigma and style of some Australian species of *Acacia*. Austral. J. Bot. 29: 733–745.

_____ & _____. 1985. Self-incompatibility in the nitrogen-fixing tree, *Acacia retinodes:* quantitative cytology of pollen tube growth. Theor. Appl. Genet. 69: 481–488.

Knox, R. B. 1984. Pollen–pistil interactions. Pp. 508–608 *in* H. F. Linskens & J. Heslop-Harrison (editors), Cellular Interactions. Encyclopedia of Plant Physiology, New Ser. 17. Springer-Verlag, Berlin.

_____ & J. Kenrick. 1983. Polyad function in relation to the breeding system in *Acacia*. Pp. 411–418 *in* D.

Mulcahy & E. Ottaviano (editors), Pollen: Biology and Implications for Plant Breeding. Elsevier Biomedical, New York.

Lord, E. M. & L. U. Kohorn. 1986. Gynoecial development, pollination, and the path of pollen tube growth in the tepary bean, *Phaseolus acutifolius*. Amer. J. Bot. 73: 70–78.

Owens, S. J. 1985. Stigma structure and the pollen–stigma interaction in Caesalpinioideae–Leguminosae. Pp. 84–87 *in* M. T. M. Willemse & J. L. van Went (compilers), Proc. 8th Int. Symp. on Sexual Reproduction in Seed Plants, Ferns and Mosses, August 1984, Wageningen. Pudoc, Wageningen.

———, S. McGrath, M. A. Fraser & L. R. Fox. 1984. The anatomy, histochemistry and ultrastructure of stigmas and styles in Commelinaceae. Ann. Bot. (London) 54: 591–603.

Patel, V., J. J. Skvarla, I. K. Ferguson, A. Graham & P. H. Raven. 1985. The nature of threadlike structures and other morphological characters in *Jacqueshuberia* pollen (Leguminosae: Caesalpinioideae). Amer. J. Bot. 72: 407–413.

Polhill, R. M., P. H. Raven, & C. H. Stirton. 1981. Evolution and systematics of the Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Randell, B. R. 1970. Adaptations in the genetic system of Australian arid zone *Cassia* species (Leguminosae, Caesalpinioideae). Austral. J. Bot. 18: 77–97.

Sedgley, M. & M. A. Blesing. 1982. Foreign pollination of the stigma of watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai). Bot. Gaz. (Crawfordsville) 143: 210–215.

POLLEN–PISTIL INTERACTIONS IN LEGUMINOSAE (MIMOSOIDEAE)

Josephine Kenrick[1] and R. Bruce Knox[1]

Abstract

This review aims to provide an understanding of the structural and physiological systems controlling pollination and the process of fertilization in *Acacia* and other mimosoid legumes. While mimosoids vary considerably in details of their inflorescence arrangements, the flower heads show relatively little variation, comprising prominent anther filaments and a style bearing a cup- or funnel-shaped stigma. The flowers of *Acacia* are generally protogynous. The stigmas are of the wet nonpapillate type and are receptive from the moment the petals open and the style begins to extend. The receptive stigma is characterized by acquisition of a copious exudate comprising saturated and unsaturated lipids, free fatty acids, carbohydrates, proteins, and polyphenols. The pollen of mimosoids is frequently compound, comprising 4–64 grains held within a polyad. Each grain is bicellular and is unusual in *Acacia* in possessing membrane labyrinths containing coated vesicles at each aperture. A postpollination exudate is produced on *Acacia* stigmas within 30 minutes of pollination and contains up to 1.3 μl of a nectarlike fluid. The polyads float on this fluid, and by surface tension, a single polyad generally fits neatly into the cup. Following germination the tubes emerge from the lower side of the polyad and induce progressive necrosis of the stigma and stylar transmitting cells. Pollen tube growth rate is similar following self- and cross-pollination in *A. retinodes*, and tubes grow into the ovule. Fecundity in mimosoids and the barriers to fertilization that have been found in *Acacia* are considered in terms of pollen viability and its estimation, the effect of environmental factors on fruit and seed set, and homomorphic self-incompatibility. The estimation of self-incompatibility using the index of self-incompatibility is presented for several *Acacia* species and other mimosoids. The majority of self pollen tubes are arrested in the nucellus of the ovule in *A. retinodes* and *A. terminalis*, and only a small percentage of self gametes form zygotes, so that no seed is set.

I. Introduction

The mimosoids are nitrogen-fixing trees and shrubs with a largely pantropical distribution. In Australia the subfamily is distributed from the equator to 43° south latitude. The continent is a major center of diversity for *Acacia*, with 900 of the more than 1,200 species worldwide occurring there. This radiation has apparently occurred since the Gondwana separation (Barlow, 1981). Since much of the work on pollen–stigma interactions has been carried out on *Acacia*, we make no apology that much of this paper is concerned with this genus.

The mimosoid flower presents a particular challenge in plant reproductive biology. On the one hand, this is because of its unusual features: pollen often in complex compound structures, polyads (Guinet, 1969, 1981); the long narrow style and stigma, often small enough to be viewed in cross section on a single grid by transmission electron microscopy (Kenrick & Knox, 1981a); and presence or absence of anther glands and floral nectaries. On the other hand, the flowers are relatively simple in their structural organization. Yet the compound flower heads show remarkable adaptations for pollination by insects, birds, and bats (Arroyo, 1981; Hopkins, 1984; Knox et al., 1985).

Here we review the present state of knowledge of pollen–pistil structure and interactions, with a view to stimulating further research into the remarkable reproductive biology of mimosoids.

[1]School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia.

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Monogr. Syst. Bot. Missouri Bot. Gard. 29:127–156. 1989.

TABLE 1. Floral characters of six Australian species of *Acacia*. One species, *A. terminalis*, is represented by two populations; all from natural populations in national parks in Victoria. Mean numbers (and range) are given.

Species (Number of trees sampled)	Floral characters					
	Flowers/head	Ovules/ovary	Style length (in mm)	Anthers/flowers	Grain/polyad	Polyads/head
A. mearnsii (7)	29 (20–39)	12 (10–15)	4 (3–5)	42 (27–64)	16	9,744
A. myrtifolia (3)	3 (1–3)	7 (6–8)	4 (3–6)	537 (323–645)	8	12,888
A. paradoxa (7)	39 (29–48)	8 (5–10)	4 (3–5)	91 (61–129)	8	29,392
A. pycnantha (8)	44 (23–56)	10 (8–12)	4 (3–5)	34 (21–48)	16	11,968
A. retinodes (8)	25 (18–34)	13 (10–15)	2 (2–3)	66 (34–91)	16	13,200
A. terminalis						
montane (4)	10 (7–12)	12 (10–14)	4 (3–6)	124 (84–160)	16	9,920
valley (5)	8 (6–11)	11 (7–14)	5 (2.5–6)	149 (30–214)	16	9,536

II. Floral Biology

In gross morphology, the globose or spicate flower heads of most mimosoids are at first sight remarkably similar. The characteristic feature is the prominence of the anther filaments which, in many cases, is responsible for the size, shape, and color of the head. The terminology for floral structures used in this review follows the widely accepted practice of designating the *inflorescence* as the floral shoot, which may be a raceme or panicle. In contrast, the *flower head* is used to describe the globose or spicate clusters of flowers. The flower head has also been treated as a shortened raceme (Robbertse, 1974).

A. Female Reproductive Structures

1. The Characters of the Flower Head

Mimosoids vary considerably in flower head arrangement and in details of floral morphology. Species differ in having globose or spicate flower heads; in whether heads are sessile or pedunculate; and in whether they are single, paired, several in the leaf axil, or arranged in racemes or in panicles of heads. Variation occurs in petal, sepal, bract, and bracteole morphology and in the numbers of floral parts as well as in the sequence in which heads flower within racemes and on branches; but generally the flowers in a compound head flower synchronously.

The number of flowers per head varies widely. In the small sample of *Acacia* species shown in Table 1, this character varies from as low as 3 in *A. myrtifolia* to 44 in *A. pycnantha*. Each flower has a single pistil, bearing 7–13 ovules (Table 1), and style length varies from 2 to 5 mm. Even more striking is the variation in number of anthers per flower, which varies from 34 in *A. pycnantha* to 537 in *A. myrtifolia*. Note that this character shows the reciprocal relationship to number of flowers per head. These data suggest that despite genetically controlled changes in flower number, anther number has changed to maintain a high level of anthers per head. It seems likely that there is a link between genes controlling these floral characters and some selective advantage in maintaining a high pollen output from each flower head. Such data confirm Arroyo's (1981) conclusion that the flower head is the pollination unit in mimosoids.

Variation is recorded in the kinds of flowers within the head. Specialization of flowers in different positions within the head in *Parkia* has been associated with bat-pollination (Hopkins, 1984, 1986). In *Albizia lebbek* the terminal flower of each head bears a floral nectary, which is

absent in the other flowers of the same head (Maheshwari, 1931). Some flowers are only male, while others are hermaphrodite. *Acacia suaveolens* shows 17–50% of male flowers per head (Morrison, 1986). In *A. karroo,* the hermaphrodite flowers are located distally in each head (Gordon-Gray & Ward, 1975), while in *Neptunia* the lower flowers are male or sterile (Geitler, 1927; Tucker, 1984a; Windler, 1966).

2. *Floral development and the female phase*

There have been remarkably few studies of mimosoid floral development (Derstine & Tucker, 1985; Dnyansagar, 1955; Tucker, 1984b, this volume). Recently, a considerable amount of information has been obtained for Australian species of *Acacia.* Eight stages of floral development in *A. retinodes* have been defined empirically according to morphological features (Knox et al., 1989; Fig. 1A). This classification is accurate and reproducible, making the sampling of different stages of anthers and pistils possible. The stigmas of mimosoids are generally classified as wet, nonpapillate (i.e., type WNP in the scheme of Heslop-Harrison, 1981).

The stigma morphology of the Mimosoideae varies from funnel-shaped in *Dinizia,* tubular in *Entada* and *Prosopis,* to narrow porate in *Adenanthera* and *Piptadenia* (Lewis & Elias, 1981). In *Acacia,* the stigma morphology appears to be remarkably uniform, and the receptive site is saucer-shaped with a heavily cutinised rim.

Data concerning the nature of stigma receptivity are available for an Australian species of *Acacia,* where ultrastructural and cytochemical studies have been carried out. In *A. retinodes* the stigma exudate arises during development from several sequential phases of secretion (Knox et al., 1989). At maturity, the exudate contains proteins, carbohydrates, lipids, and phenolic compounds. Esterase activity, a marker of receptivity, is present from anthesis and increases throughout the flowering period (Kenrick & Knox, 1981a).

There are distinct female and male phases of flowering in *Acacia,* namely stages 6, 7, and 8 in Figure 1. Apart from the presence of the exudate, interesting developmental changes associated with receptivity in female phase stigmas can be seen at the ultrastructural level (Fig. 2). There is the appearance of wall ingrowths or flanges in the stigma surface cells of *Acacia.* It may be that these ingrowths act as transfer cell walls for transport of components into or out of the stigma cells. The flanges may also provide increased wall surface area across which secretion can pass to the stigma surface, and they increase in size and prominence in male phase stigmas. Male phase stigmas differ strikingly in the loss of cellular membranes, especially the plasma membranes that were well defined in the female phase (Knox et al., 1989). This suggests that the stigma cells at this phase are no longer viable. Receptivity based on seed set is highest at stage 6, falls sharply in stage 7, to zero at stage 8 (Knox et al., 1989).

Style length varies in different species (Table 1) and even in different populations of the same species, e.g., *Acacia terminalis.* Style length may be related to pollination vector (Kenrick et al., 1987). The epidermal cells of the mature style are remarkable in being four times larger in diameter than the inner cells, covered by a thick cuticle on the outer wall, and their cytoplasm is almost filled with polyphenol vacuoles (Kenrick & Knox, 1981a). Cells of the transmitting tissue are loosely packed and filamentous, about four times longer than their diameter. The transmitting cells are sited within a mucilaginous extracellular matrix that stains positively for proteins and carbohydrates. There is no entasis as found in certain papilionoid styles (Heslop-Harrison & Heslop-Harrison, 1982). A single vascular trace terminates below the stigmatic zone about four cells beneath the stigmatic surface.

The ovary is pod-shaped, containing 2–14 ovules in a range of species (Tables 2, 3). Ovules are attached to the placenta in a double row by an often large fleshy aril and long funicle. The tips of the nucellus rest among thick-walled trichomes in the ovarian cavity in *A. retinodes* and some other species.

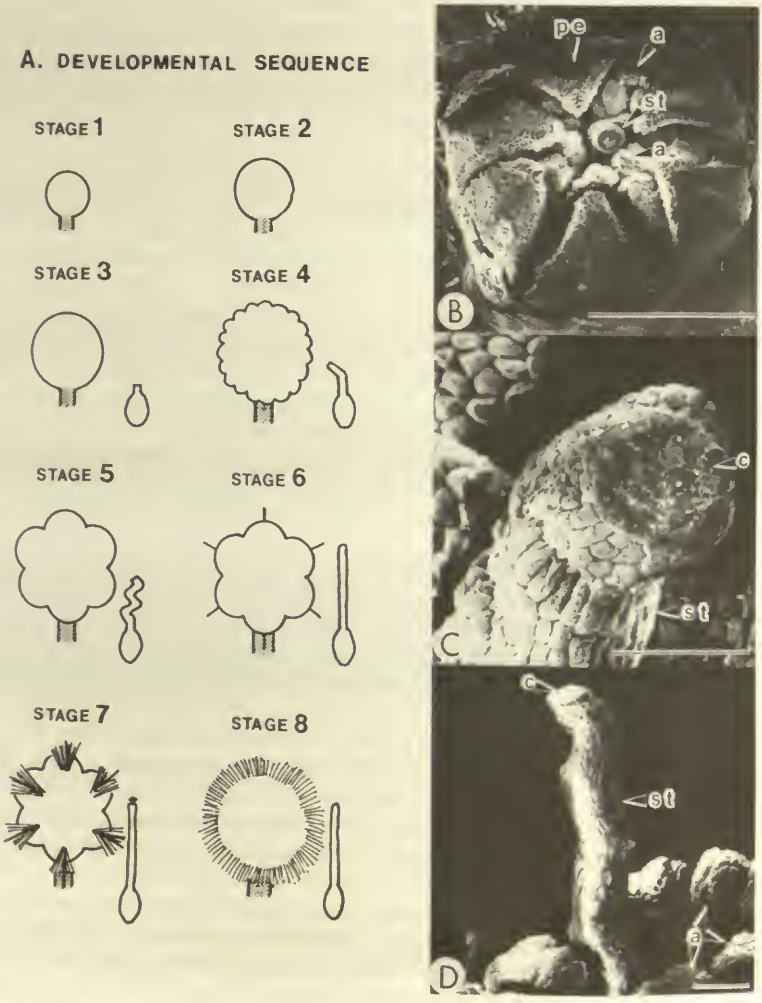

FIGURE 1. Floral development in *Acacia retinodes*.—A. Diagram of eight stages. *Stage 1*. Meristematic: flower head bud not much larger than peduncle and covered by bracteoles. *Stage 2*. Meristematic: flower head bud round and green; bracteoles almost cover the bud; some floral primordia visible, with the style initiated as growth center at tip of ovary. *Stage 3*. Flower head bud larger, style has grown to form short rodlike protuberance. Flower buds still protected by bracteoles. *Stage 4*. Flower head bud greed, still partly covered by bracteoles; style has a single fold. *Stage 5*. Flower head comprises conspicuous yellow flower buds that have swollen to leave the bracteoles as small hard structures subtending individual flower buds; style has multiple folds. *Stage 6*. Petals have begun to open; stigma and tip of style exposed (*early female phase*, Fig. 1B); bracteoles hardly visible under the petals. *Stage 7*. Mature flowers with style fully extended; filaments beginning to extend and anthers nondehiscent (*female phase*, Fig. 1C). *Stage 8*. Fully mature flowers; stamens exserted often obscuring styles; anthers dehiscent (*male phase*); bracteoles eclipsed by corolla and androecium.—B–D. Scanning electron micrographs of flowers of *Acacia retinodes* at female phase (from Knox et al., 1989). B. Early female phase at anthesis, petals (pe) parting to reveal stigma style (st) and anthers (a). C. Stigma and style (st) extending; note saucer-shaped stigma disc (c) filled with exudate. D. Flower at late female phase showing elongate stigma (c) and style (st) with undehiscent anthers (a). Scale bars: A = 0.5 μm, B = 60 μm, C = 70 μm.

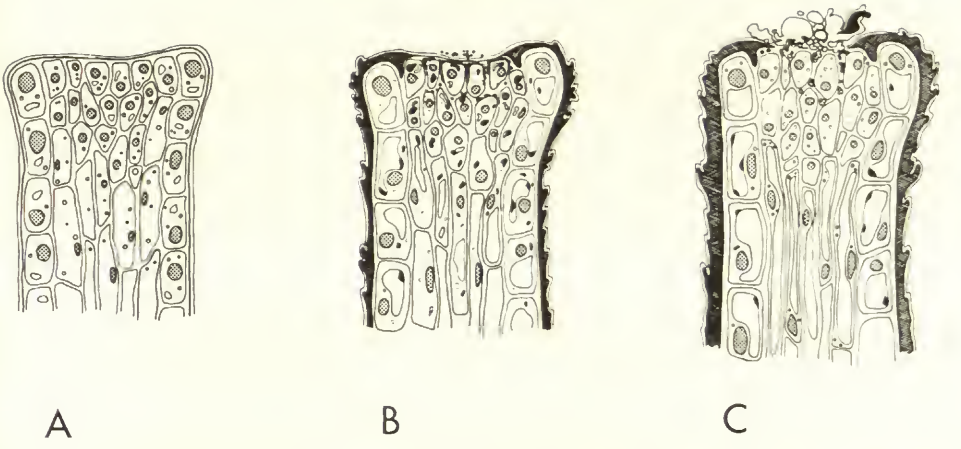

FIGURE 2. Diagrams showing the structure and development of the stigma of *Acacia retinodes* and acquisition of its copious exudate (from Knox et al., 1989).—A. Presecretory stigma from meristematic flower head bud (Stage 3). Nuclei are cross-hatched, and the surface is covered by a primary cuticle.—B. First secretory period in flower head bud prior to opening (Stage 4). The stylar and stigmatic rim cells are covered by a thick secondary cuticle (hatched) and the cells are vacuolate. Storage protein/phenolic compounds are indicated in cytoplasm (black bodies).—C. Second secretory period just prior to flower opening (Stage 5). A massive lipid secretion is present in intercellular spaces of stigma surface.

In the mature unfertilized ovary of *A. retinodes*, the ovules are amphitropous, crassinucellate, and bitegmic. There is little development of the integuments, and they cover only the chalazal/antipodal half of the embryo sac, so that there is no true micropyle. An area of three or four layers of tightly packed nucellar cells protects the embryo sac (see III C3, 4). It is through this tissue that the pollen tubes have to pass. The integuments develop after anthesis and the ovules become anatropous.

B. MALE REPRODUCTIVE STRUCTURES

In the male phase the abundant stamens dominate the appearance of the mimosoid flower head. While anther number is variable between species, anther shape and structure vary little, except for the presence of a terminal anther gland (sessile or stalked) common in some species of Parkieae (Elias, 1981) and Mimoseae (Lewis & Elias, 1981), especially in *Acacia* spp. from Africa (Robbertse, 1974) and some from Asia (e.g., *A. catechu* [Rao, 1954] and *A. nilotica* [Sinha, 1971]). Development of the anther gland was studied by Rao (1954), who implicated the gland in pollen presentation of *A. catechu*. The glands are typical secretory structures having cells with dense cytoplasmic contents. Their cytochemistry needs to be explored to determine the nature of any secretions that may be specific adaptations for pollination.

Vishnu-Mittre & Sharma (1962) found that the presence or absence of anther glands is not associated with the polyad or monad state in the Mimosoideae. This structure is not generally present on Australian *Acacia* species, although there are exceptions— for example, *A. auriculiformis* (Dnyansagar, 1958) and *A. bidwillii*, in which stalked glands are present on some of the anthers in each flower (Fig. 3).

The entire anther epidermis in *Acacia retinodes* shows a positive reaction for osmophores (the probable source of floral fragrances) at the male phase but not the female phase of flowering

TABLE 2. Relationships between number of grains in polyad to seed set in various species of *Acacia* according to the classification of Pedley (1978–1979). (From Kenrick & Knox, 1982.)

(a) Australian species

Species name	Polyad grain number	Ovule number	Polyad/ovule ratio	Pod seed number	
				Mean	Maximum
Section Acacia					
1. *A. bidwillii*	16[a,g],32[k]	10[a]	1.6	ND	7
Section Botryocephalae					
2. *A. baileyana*	16[g,n]	12[a]	1.3	7.6[a]	12[a]
3. *A. dealbata*	16[g]	12[a]	1.3	ND	ND
4. *A. decurrens*	16[g]	12[a]	1.3	6.0[j]	ND
5. *A. mearnsii*	16[g]	14[a,j]	1.1	5.1[a], 6.8[i]	12[a],14[i]
6. *A. spectabilis*	16[g]	10[a]	1.6	2.8[a]	9[a]
Section Phyllodineae					
7. *A. dorothea*	16[a]	8[a]	2.0	ND	3[c]
8. *A. hispidula*	8[a]	2[a]	4.0	ND	2[c]
9. *A. howittii*	16[f]	10[a]	1.6	2.4[a]	5[a]
10. *A. lineata*	8[g]	8[a]	2.0	ND	ND
11. *A. paradoxa*	8[g]	10[a]	0.8	3.4[a]	8[a]
12. *A. podalyriifolia*	16[g]	12[a]	1.3	4.2[a]	11[a]
13. *A. rupicola*	8[f]	ND		3.5[a]	8[a]
14. *A. suaveolens*	16[f]	5[o]–7[a]	3.2–2.6	2.9[a]	7[a]
15. *A. subulata*	16[g]	12[a]	1.3	5.5[a]	11[a]
Section Lycopodifoliae					
16. *A. baueri*	4[k]	1–3[a]	4.0–1.3	1.5[a]	3[a]
Section Plurinerves					
17. *A. melanoxylon*	16[g]	14[a]	1.1	6.9[a]	13[a]
18. *A. rigens*	16[f]	9–13[a]	1.7–1.2	7.3[a]	12[a]
Section Juliflorae					
19. *A. longifolia*	16[f]	10–12[o]	1.6–1.3	ND	12[l]
20. *A. longifolia* var. *sophorae*	16[g]	14[a]	1.1	6.9[a]	13[a]

(b) Species from other continents

Species name	Polyad grain number (1)	Maximum pod seed number (2)	Ratio (1)/(2)
African and Asian species			
A. albida	32[g]	21[d]	1.5
A. ataxacantha	16[g]	8[d]	2.0
A. borleae	16[e]	8[d]	2.0
A. burkeii	16[e]	5[d]	3.2
A. caffra	16[e]	10[d]	1.6
A. davyi	16[e]	12[d]	1.3
A. erioloba	32[e]	24[d]	1.3
A. exuvialis	16[e]	7[d]	2.3
A. farnesiana	8[m], 16[g], 32[g]	11[r] (ovule no. = 14[m])	0.7–2.9
A. gerrardii	16[e]	10[d]	1.6
A. grandicornuta	16[e]	12[d]	1.3
A. haematoxylon	16[e]	14[d]	1.1
A. hebeclada	16[e]	15[d]	1.1
A. hereroensis	16[e]	8[d]	2.0

TABLE 2. Continued.

Species name	(b) Species from other continents		
	Polyad grain number (1)	Maximum pod seed number (2)	Ratio (1)/(2)
A. mellifera	16[e]	5[d]	3.2
A. nebrownii	16[e]	6[d]	2.7
A. nigrescens	16[e]	6[d]	2.7
A. nilotica	16[e]	15[d]	1.1
A. pennata	16[e]	16[d]	1.0
A. permixta	16[e]	6[d]	2.7
A. rehmanniana	16[e]	11[d]	1.5
A. senegal	16[e]	6[d]	2.7
A. swazica	16[e]	10[d]	1.6
A. tortilis	16[e]	14[d]	1.3
A. xanthophloea	16[e]	9[d]	1.7
African and West Indian species			
A. emoriana	ND	8[q]	—
A. gregii	16[g]	11[h]	1.5
A. insulae-iacobi	ND	15[t]	—
A. rorudiana	ND	16[t]	—
A. tortuosa	16[g]	13[b]	1.2
A. wrightii	16[g]	7[r]	2.3
Pacific Island species			
A. koa	16[s]	12[p]	1.3

Correlation of polyad grain number and pod seed number tested by Kruskal-Wallis nonparametric one-way analysis of variance, X^2 3d.f. = 10.12, $p > 0.02$ (Siegel, 1956). Polyad grain number and pod seed number are taken from the following sources. [a]Data collected from cultivated specimens for species 2, 4, 6, 9, 10, 13, and 15; from herbarium specimens at New South Wales for species 7, 8, and 16; and others from natural habitats: 1 from Gayndah, Queensland; 3 from Coranderrk, Victoria; 5, 11, and 20 from Somers, Victoria; 12 from Pimpama and Mogill State Forest, Queensland; 14 from French Island; 17 from Wombat State Forest; 18 from roadside, Nhill, Victoria. [b]Adams, 1972. [c]Armitage, 1977. [d]Carr, 1976. [e]Coetzee, 1955. [f]Cookson, 1954. [g]Guinet, 1969, and personal communication. [h]Jepson, 1951. [i]Moffett, 1956. [j]Moffett & Nixon, 1974. [k]Mueller, 1887–1888. [l]Murray et al., 1978. [m]Narasimhachar, 1948. [n]Newman, 1934a. [o]Newman, 1936. [p]Rock, 1913. [q]Sargent, 1922. [r]Sargent, 1947. [s]Selling, 1947. [t]Wiggins & Porter, 1971.

(Bernhardt et al., 1984; see Bernhardt, this volume) and is believed to be the principal source of scent in the mature flowers.

1. Polyad structure and mode of anther dehiscence

The anther of *Acacia* is bilobed and set terminally on the filament. Each lobe has four separate loculi paired one above the other. The inner surface of each loculus is lined with closely packed, almost spherical orbicules (Fig. 4B), which are held together at their bases to form a "culture sac" in which a single compound pollen grain or polyad develops (Kenrick & Knox, 1979). In *Parkia biglandulosa* there are many polyads arranged in a vertical row, but each is enclosed within its own spore sac (Dnyansagar, 1954).

In most Australian *Acacia* species, the polyad is a composite of 8, 12, or 16 grains arranged to form a biconvex disc that is two grains thick in the center, with the remaining grains arranged singly on the periphery. The mature polyad reflects the original distribution of the sporogenous cells and of the resulting tetrads of microspores (Wodehouse, 1935). Pollen grains of Australian species of

TABLE 3. Relationships between number of grains in polyad (or pollen) to seed number in selected mimosoid species.

Species name	Tribe[a]	Polyad grain	Ovule number	Pod seed number ratio	Polyad/ ovule
Calliandra haematocephala	I	8[1]	8[1]	5–6	1.00
Desmanthus illinoensis	M	monad[2]	—	1–7[3]	—
Dichrostachys cinerea	M	8–16[4]	8–15[5]	—	—
Inga brenesii	I	34[6]	21[6]	—	1.60
I. densiflora	I	31[6]	18[6]	—	1.74
I. longispica	I	16[6]	13[6]	—	1.26
I. mortoniana	I	16[6]	17[6]	—	0.95
I. oeastedina	I	31[6]	18[6]	—	1.73
I. punctata	I	23[6]	19[6]	—	1.20
I. quarternata	I	22[6]	20[6]	—	1.10
Leucaena glauca	M	monad[2]	—	15[7]	—
L. leucocephala	M	monad[2]	—	20–27[8]	—
Neptunia oleracea	M	monad[2]	6–10[11]	4–8[9]	6–10
N. triquetra	M	monad[2,10]	2–8[10]	4–8[9]	2–8
N. lutea	M	monad[2]	—	4–9[9]	—
N. monosperma	M	monad[2]	—	1(–2)[9]	—
Parkia biglandulosa	P	12[3], 16[5]	10–20[5]	—	1.9
P. bicolor	P	16–24[2]	—	10–25[12]	—
P. filicoidea	P	16–20[2]	—	10–21[12]	—
P. madagascariensis	P	16–20[2]	—	12[12]	—
Prosopis reptans	M	monad[2]	—	3–4[13]	—
P. caldenia	M	monad[2]	—	18–20[13]	

[a]I = Ingeae, M = Mimoseae, P = Parkieae.
[1]Nevling & Elias, 1971.
[2]Guinet, 1969.
[3]Latting, 1961.
[4]Vishnu-Mittre & Sharma, 1962.
[5]Dnyansagar, 1954.
[6]Koptur, 1984.
[7]Hutton & Gray, 1959.
[8]Wirjodarmodjo & Wiroatmodjo, 1983.
[9]Windler, 1966.
[10]Dnyansagar, 1952.
[11]Singh & Shivapuri, 1935.
[12]Hopkins, 1983.
[13]Solbrig & Cantino, 1975.

Acacia possess circular depressions on the outer surfaces of the polyad, probably from contact with the orbicules during development (Kenrick & Knox, 1979). A deep rectangular pseudofurrow on the exposed surface of each grain is characteristic of Australian species (Coetzee, 1955; Guinet, 1981). A complete review of these surface characters of *Acacia* pollen is given by Guinet (1986).

At early tetrad stage, when the microspores of *Acacia* are held within the callose walls, cytoplasmic connections are visible between cells (Kenrick & Knox, 1979; Knox & McConchie, 1986). However, these channels close during early microspore development, being replaced by wall bridges that are initiated as the exine is laid down.

The primexine, the layer that initiates patterns formation of the exine of *Acacia*, is laid down external to the plasma membrane of the microspores on the outer tangential walls at tetrad period (Knox & McConchie, 1986). A very thin granular layer is also present in the periplasm around the

FIGURE 3. Anther of *Acacia bidwillii* showing the stalked terminal gland on anther.

inner faces of the microspores. This marks the site where the inner layer of the exine, the nexine, will be formed. Later, a thin electron-dense layer appears all around the grains, ramifying into the apertures as white-line lamellations or trilaminar plaques apparently coated with sporopollenin. Nexine bridges occasionally cross the callose wall between adjacent microspores. Subsequently, at the time equivalent to spore release in monad systems, the callose wall has undergone autolysis on the outer surface of the polyad but remains within and between the uninucleate microspores. Each microspore has become surrounded by a well-developed nexine, and on the outer surface by a thin tectum covering a granular layer.

When the uninucleate microspores have developed an exine that has two layers—the tectum and the nexine of about the same thickness—a transformation occurs within the intercellular spaces. The surface of the internal radial walls has become coated with intercellular strands (Knox & McConchie, 1986). These may be similar to the pollen-connecting threads reported by Waha (1984) from SEM studies.

In the maturing polyads, a great deal of electron-dense fibrillar material accumulates in these intercellular spaces, derived from the tapetum, which may function as a cement to strengthen the bonds linking the grains together (Barth, 1965; Guinet & Lugardon, 1976). Interestingly, when the polyad germinates, these same intercellular sites provide the route for pollen tube growth through

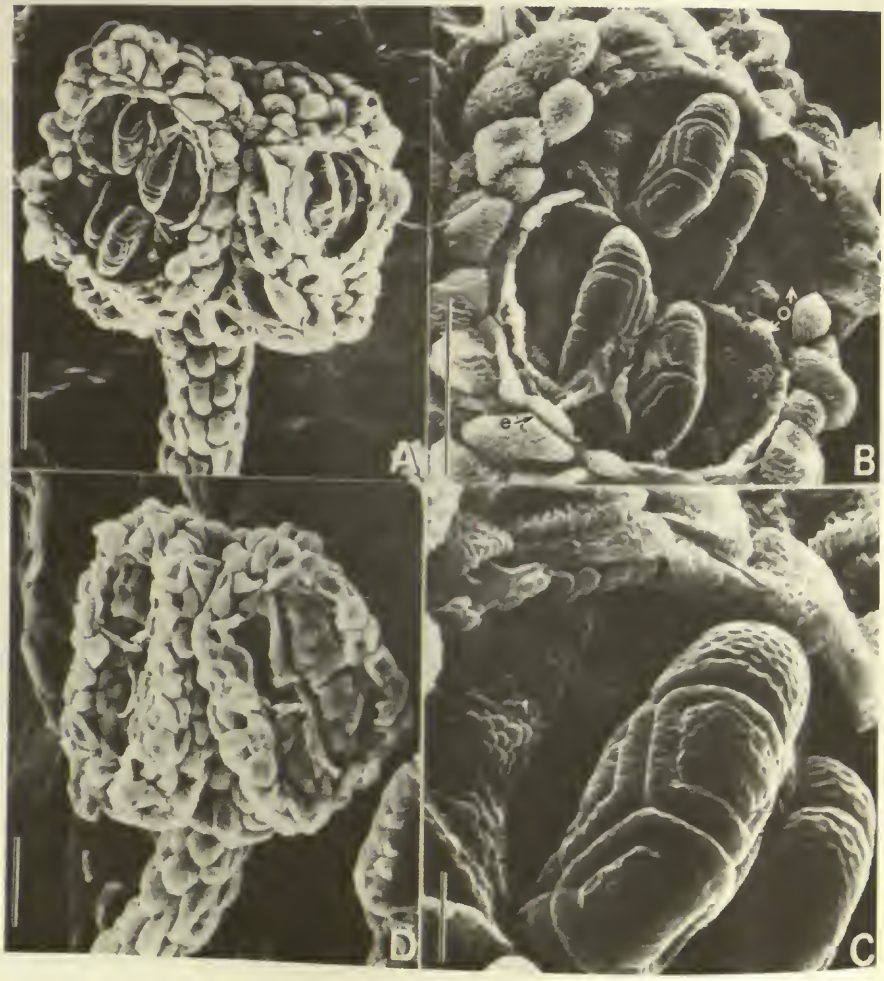

FIGURE 4. Scanning electron micrographs showing dehiscence of polyads from anthers of *Acacia subulata* (from Kenrick & Knox, 1979).—A. Anther at dehiscence showing the polyads emerging.—B. Detail at higher magnification showing sac lined with orbicules (o), outer ridged epidermal cells (e).—C. Detail of polyad and orbicules.—D. Anther after polyad dehiscence. Scale bars: A, B, D = 50 μm; C = 10 μm.

the polyad to the stigma surface. The strength of cohesion in the polyad varies; for example, in *A. baileyana* polyads, the grains frequently split apart during pollen germination (Newman, 1934b; Kenrick, unpublished data), whereas this has not been observed in *A. retinodes, A. subulata, A. genistifolia,* or *A. terminalis.*

Associated with pollen maturation are striking changes in the generative cell that occur as late as the final 24 hours prior to pollen dehiscence. At female phase, the generative cell is attached to the plasma membrane of the vegetative cell (McCoy & Knox, 1988). By male phase, the generative cell elongates into a lens shape with a scalloped surface, lying centrally across the mature grain, but remains attached to intine wall ingrowths near one of the germinal apertures. These changes are assumed to be associated with the progressive dehydration of the grains.

Further progress in understanding these remarkable changes in generative cell ontogeny will depend on three-dimensional reconstruction of serial thin sections by transmission electron microscopy. Such technology has already revealed the remarkable spindle shape and tails of the generative cell of *Rhododendron* (Ericaceae), which is unusual in that one of the tails is attached to an intine wall ingrowth in the mature pollen (Theunis et al., 1985). More sophisticated interactive computer graphics techniques have been applied to generative cell ontogeny in *Brassica* (Cruciferae) (Tuohy et al., 1987).

Associated with these changes to the generative cell, membrane labyrinths are initiated at the germinal apertures at female phase. By male phase, these are filled with coated vesicles that are assumed to play a role in protein transport during tube emergence at germination (McCoy & Knox, 1988).

Anther dehiscence occurs through the stomium, a file of thin-walled cells which split vertically, assisted by an endothecium with ridges of wall thickening which forces the polyads out of the orbicule-lined sac (Fig. 4), leaving the sac partly inverted (Kenrick & Knox, 1979).

2. *Polymorphism for polyad grain number*

Polyad grain number is constant for most *Acacia* species but can be variable in others where the polymorphism is expressed in individuals or populations. Variation within plants and populations can arise from hybridization (Ali & Qaisar, 1980; Guinet, 1982; Leach & Whiffen, 1978). About 90% of Australian *Acacia* species have 16-grain polyads, other species have 8- or 12-grain polyads, while *A. baueri* has tetrads. In most sections of the genus less than 5% of species have 8 grains (Guinet, 1969, 1981); however, in Phyllodineae and Alatae frequencies are higher, 19% and 29% respectively (Guinet, pers. comm., 1982). Grain numbers higher than 16 (up to 64) occur in some African and Asian species of *Acacia* (Table 2) and in other mimosoids (Table 3).

The polyad polymorphism arises through developmental control of the numbers of sporogenous cells which form microsporocytes that undergo meiosis (Fig. 5) to produce the microspores. The microspores develop into bicellular pollen grains (Newman, 1934a; Narasimachar, 1948; Dnyansagar, 1955). Thus the polyad represents all the products of meiosis within a single loculus, arranged in such a way that the members of each tetrad can be identified. In *A. baueri*, there is likely to be a single microsporocyte which forms a tetrad of microspores (Fig. 5a); in the species with 8-grain polyads, there is a single round of mitosis of the sporogenous cell to form two microsporocytes (Fig. 5b); in species with 12-grain polyads, the developmental sequence has not yet been studied, but it presumably involves at least two rounds of mitosis to form three microsporocytes (Fig. 5c), while in species with 16-grain polyads there are two rounds of mitosis to form four microsporocytes (Newman, 1933; Kenrick & Knox, 1979; Knox & Kenrick, 1983) (Fig. 5d).

There is a correlation between grain number in the polyad and maximum pod seed number in many species of *Acacia*. Maximum pod seed number is obtained by scoring pods from individual trees in natural populations, and Table 2 show results for available species. The maximum seed number does not exceed the polyad grain number, suggesting a link in genetic control (Kenrick & Knox, 1982). One character limiting seed number that is likely to be under genetic control is ovule number.

Control of the number of ovules per pod limits seed numbers. Polyad grain number is correlated with ovule number (Table 2). In one species, *A. baueri*, the polyad grain number/ovule number varies from 4/1 to 4/3; in *A. hispidula* it is 8/2; while in *A. mitchellii* and *A. paradoxa* it is 8/10. In species with 16-grain polyads the ratio varies widely. *A. suaveolens* is 16/7; *A. bidwillii*, *A. spectabilis*, and *A. howittii* are 16/10; while most species are 16/12 or 16/14. A surprising feature of ovule number is that it may vary between individuals in a species: 1–3 in *A. baueri*, 5–7 in *A. suaveolens* and 9-13 in *A. rigens* (Kenrick & Knox, 1982).

We conclude that while maximum pod seed number generally seems to be less than or equal to

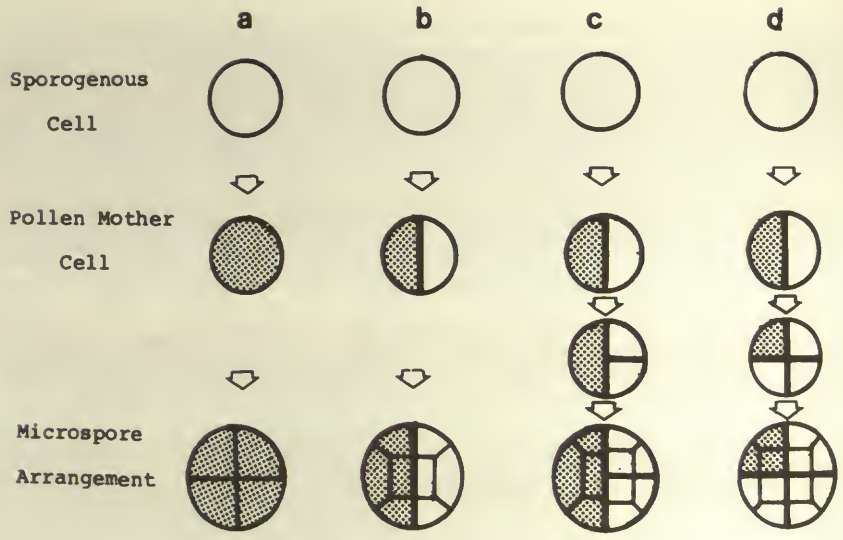

FIGURE 5. Diagram showing origins of (a) 4-, (b) 8-, (c) 12-, and (d) 16-grain polyads in Australian species of *Acacia;* (a) is hypothetical, others are based on observations of (b) *A. myrtifolia,* (c) *A. drummondii,* and (d) *A. subulata* (from Knox & Kenrick, 1983).

the polyad grain number, ovule number differs, being less than or even slightly greater than the grain number. What is the selective advantage in reproduction for this apparent correlated polymorphism linking polyad grain number with ovule number and seed set? At first sight, the mechanism provides for the potential to set a full pod of seeds following a single pollination event, as noted by Elias (1974). The polyad of *Acacia* may be considered analogous to the pollinia of epidendroid–vandoid orchids and the polyad of certain Asclepiads in providing a compound pollination unit for transport by specific pollinators (Knox & Kenrick, 1983; Bernhardt, this volume).

III. CONTROL OF FERTILIZATION

A. POSTPOLLINATION RESPONSES

A visible postpollination exudate is produced within 30 minutes of pollination in a number of *Acacia* species when flowers are maintained in a humid environment (Table 4; Kenrick & Knox, 1981b; Marginson et al., 1985). For example, in 90 minutes at 20°C and 100% humidity, *A. wattsiana* stigmas can produce a surface droplet of up to 1.3 microlitres (Marginson et al., 1985). Production of the exudate can be triggered in response to soluble material on the surface of intraspecific, interspecific, and even intergeneric pollen. There is no response to damage, glass beads, yeast cells, or fixed chicken cells (Marginson et al., 1985) nor to pollen that has been washed in water or ethanol. The function of the postpollination exudate may be to provide a fluid for hydration and germination, and it may also serve to orientate a polyad into a central position within the stigma cup. Such a relationship is characteristic of an effective pollination, where there is a high proportion of single polyads per stigma (Knox & Kenrick, 1983).

Polyads germinating *in vivo* within 8 hours of pollination show a number of interesting features. The entire polyad of both *A. retinodes* and *A. genistifolia* (Fig. 6) is enveloped within an exudate, bounded by a membranelike surface bilayer. This exudate is heterogeneous after fixation and

TABLE 4. List of matings of *Acacia* spp. in which a postpollination exudate has been induced on *Acacia* stigmas within 40–90 minutes after pollination. Inflorescences were maintained on agar plates. This table is compiled from Kenrick & Knox (1981b) and Marginson et al. (1986). s = self-pollinated; × = cross-pollinated within own species.

Stigma		Pollen donor	Exudate induced
A. baileyana	s, ×	*A. baileyana*	+
	cross	*A. iteaphylla*	+
	cross	*Correa pulchella*	+
A. brownii	s, ×	*A. brownii*	+
A. dealbata	s, ×	*A. dealbata*	+
	cross	*A. drummondii*	+
	unpollinated		−
A. farinosa	cross	*A. dealbata*	+
	cross	*A. drummondii*	+
	unpollinated		−
A. gracifolia	cross	*A. dealbata*	+
A. howittii	cross	*A. melanoxylon*	+
	cross	*A. continua*	+
	cross	*Salix* spp.	+
	cross	*Ulex europaeus*	+
	cross	*Cytisus proliferus*	+
	unpollinated		−
A. iteaphylla	s, ×	*A. iteaphylla*	+
	stored	*A. iteaphylla*	+
	cross	*A. baileyana*	+
	cross	*A. rotundifolia*	+
	cross	*A. victoriae* (from bee corbiculae)	+
	cross	*Persea americana*	+
	cross	*Macadamia integrifolia*	+
	yeast cells		−
	chicken red blood cells		−
	glass beads (40 μm diam)		−
	talc		−
	Biogel PI50		−
	pollen diffusate on glass beads		+

processing for TEM but is markedly different in appearance from the receptive stigma exudate in that it is mainly electron-lucent rather than filled with lipid droplets. In pollinated stigmas, the pollen tubes emerged from the inner face of the polyad and passed down through the intercellular spaces between the stigma surface cells, apparently inducing first a dense staining of the cytoplasm followed by progressive degeneration of the stigma and stylar cells (Fig. 6). Stigma cells at the time of pollination show a marked increase in golgi activity; protein crystals were found in vacuoles of the stigma cells and also in the stigma nuclei. A conspicuous feature of early stages of pollen tube penetration is that a zone of dense uranyl acetate precipitation usually precedes the passage of the pollen tubes (Fig. 6) when uranyl acetate has been employed for en-bloc staining of the tissue. The staining is particularly dense in the cell walls, at the zone where degeneration of the stigma cells is occurring. Diffusion of uranophylic components is suggested by the zone of granules seen in both cell walls and cytoplasm in advance of the pollen tubes and the lytic zone. This suggests that holocrine secretion of the stigma cells may contribute components for tube growth.

In *Acacia*, both self- and cross-pollination results in polyad germination and pollen tube growth to the ovule (Kenrick & Knox, 1985), where self pollen tubes are arrested in self-incompatible

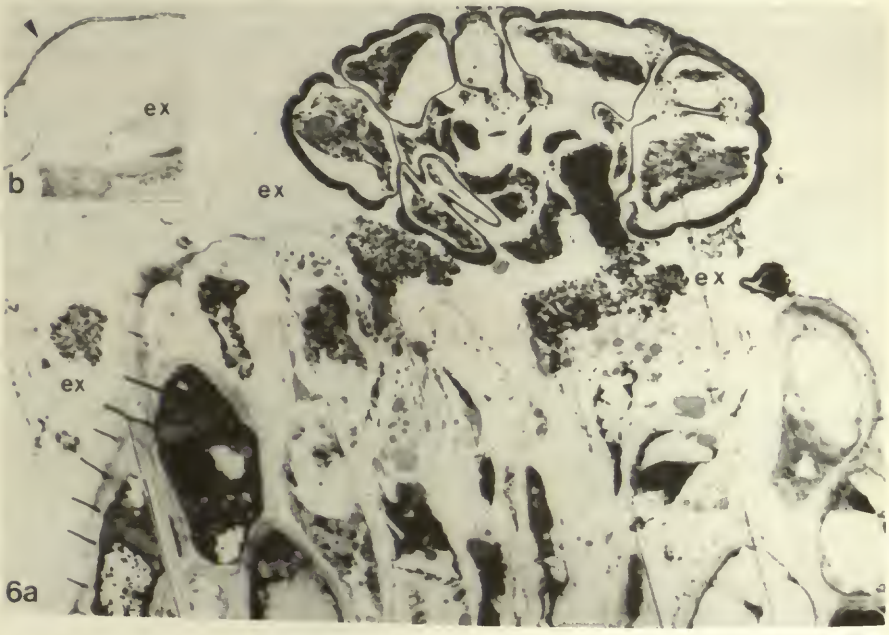

FIGURE 6. Polyad germination on the stigma of *Acacia genistifolia*, ultrastructural study.—A. Polyad on surface showing film of exudate.—B. Detail showing exudate–air interface where membranelike lipid bilayer is present.

matings (see Section III C4 below). The absence of a cuticle covering the receptive stigma exudate may favor such a mechanism and is very different from papilionoid legumes. In *Trifolium*, Heslop-Harrison & Heslop-Harrison (1983) demonstrated that the intact stigma cuticle covers the exudate, preventing pollination of untripped flowers. In *Medicago*, the situation is similar, although Kreitner & Sorensen (1984) reported that the stigmatic cuticle can be prematurely ruptured by mechanisms other than tripping.

B. REPRODUCTIVE EFFORT

This parameter estimates the allocation of total resource budget devoted to reproduction, is influenced by natural selection, and is a factor in maximizing fitness or fecundity (see Wilson, 1983). Fecundity is defined as the rate at which an individual plant contributes genes to future generations, and can be estimated by the size and mass of seeds produced by a given parent, male or female.

Mimosoid legumes have very large numbers of flowers but generally produce only a small number of ripe pods, although in most species studied there is wide variation between individual plants. For example, in self-incompatible *Prosopis flexuosa*, 10 trees produced an estimated mean of 56,000 flowers which set 145 pods, of which only 40 ripened to produce mature seed (Solbrig & Cantino, 1975). Seed production from these trees ranged from 0 to 1.25% of flowers. Even in *Leucaena*, which is reputed to have a high pod set, reproductive efficiency is surprisingly low. In self-compatible *Leucaena glauca*, Hutton & Gray (1959) reported that 0.8% of ovules in Hawaii and 0.04% in Peru produced seed. In *Leucaena leucocephala*, less than 2% of flowers develop

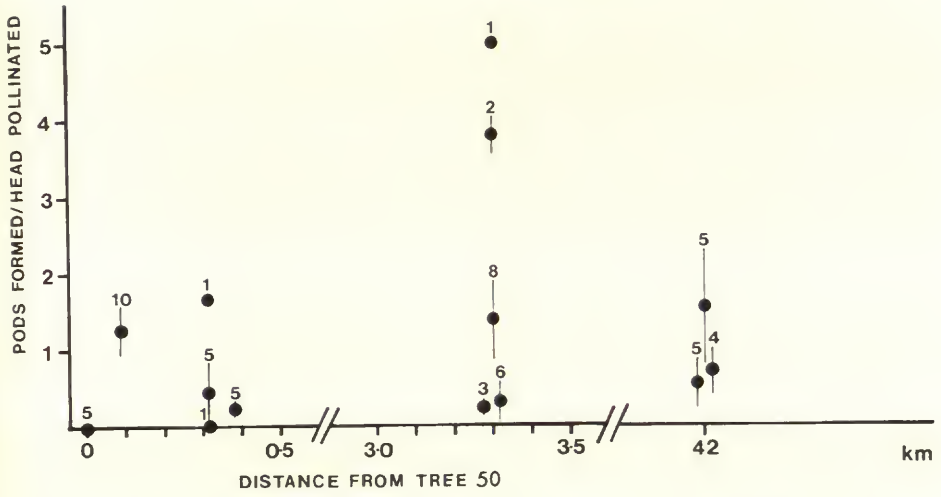

FIGURE 7. Effect of pollen from different trees of *Acacia retinodes* on pod set of a single tree, AR50. Data plotted in terms of the distance in km of pollen parents from female tree. Bars indicate ±1 SEM, and numbers indicate the number of inflorescences (flowering shoots) subjected to manipulated pollination.

into pods, a prefertilization regulative mechanism effectively restricts the formation of pods with less than a minimum number of seeds. This occurs by the inhibition of pollen germination in the stigmatic fluid when the number of grains in the stigmatic pouch is below a critical level (Ganeshaiah et al., 1986). In several species of *Acacia*, reproductive activity has been monitored during the flowering and fruiting season (Milton & Moll, 1982; Morrison, 1986). Fruit abortion is very high in *A. suaveolens* in the first five to eight weeks following fertilization, the duration of fruit development being twelve weeks (Morrison, 1986). This low reproductive effort seems to be so widespread that it is probably inherent in mimosoid systems. In breeding experiments with three populations of *Acacia retinodes*, female and male reproductive function has been explored in terms of pod set (Kenrick & Knox, in prep.). Female function was assessed by selecting one tree as father and testing its pollen on a range of females by the methods described in Section III C4 below. Some significant differences in pod set were obtained. In reciprocal experiments, one tree was selected as female parent, and pollen from a range of males was tested for pod set. Two trees showed exceptional fecundity, while the others fell within the same general range (Fig. 7). With these two exceptions, there is little effect of distance of the male from the female in these populations, sited 3.5 and 42 km apart. These data for *Acacia* do not lend support to the concept of outbreeding depression proposed by Waser & Price (1983) on the basis of pollen transfer experiments with alpine herbs.

1. Effects of temperature

Field observations of arid zone species indicate that *Acacia* flowering can be related to particular temperature and photoperiod conditions in *A. adsurgens*, *A. craspedocarpa*, *A. cuthbertsonii*, *A. kempeana*, *A. pruinocarpa*, *A. sclerosperma*, *A. tetragonophylla*, *A. victoriae*, *A.* sp. nov. [HA251274], and *A.* sp. nov. [HA 251275], even though they flowered at different seasons (Davies, 1976). However, *A. aneura* (Preece, 1971; Coaldrake, 1971; Davies, 1976) and some

other species do flower in response to rain. Davies (1976) suggested that rain induces temperature changes that mimic conditions at other times of the year.

Detailed studies of floral development in *A. pycnantha*, a species of southeastern temperate regions, tolerant of both moist and dry conditions, has shown that floral initiation may take place throughout the year, and stages of development are blocked by different environmental parameters so that flower heads open synchronously (Buttrose et al., 1981; Sedgley, 1985). Seed set in *Acacia* species is notoriously unreliable, and this is often related to environmental effects (Rao, 1954; Simpson, 1977), particularly in Australian species (Preece, 1971). Newman (1933) observed that *A. baileyana* trees growing near to sea level did not set seed. Despite abundant flowering and hand cross-pollination between trees known to be compatible, *A. retinodes* sometimes sets no pods in hot weather (>30°C).

2. *Seed set in relation to floral structure*

Although the flower heads are similar in structure, pollination studies of four *Acacia* species showed that they had floral characteristics that may influence pod set (Table 1). There is interspecific variation in number of anthers per flower and flowers per head; however, anthers/head values are of the same order of magnitude in *A. pycnantha*, *A. retinodes*, *A. myrtifolia*, and *A. terminalis*. Pod set varies with the number of pistils per head, supporting Arroyo's (1981) contention that the unit of pollination is the flower head. Pod set (under experimental conditions) was highest in *A. myrtifolia*, with only three flowers per head, and lower in species with higher pistil:anther ratios. In *A. myrtifolia*, which set some pods after hand self-pollination, self-pollination did not occur in the control bagged heads (see section below), probably due to the spacing of the flower heads and sequence of anthesis within each head. Occasional sterile flowers can be found in some heads in which the ovary is rudimentary. It is suspected that ovule development can also be arrested so that only some ovules in an ovary are mature and viable at pollination.

The floral characteristics of a range of *Acacia* species are being investigated with a view to obtaining more information on the reproductive biology of this important genus in Australia.

3. *Biennial bearing*

Biennial bearing has been suggested as a possible cause of low levels of seed set in mimosoids, and we have observed its occurrence in certain trees of *Acacia retinodes*. However, more work is required to distinguish between environmental effects and competition between ripening pods and flower head initiation.

C. REPRODUCTIVE SUCCESS

Reproductive success depends on the ability of the pollen to achieve fertilization and the continued development of the embryo. This parameter estimates the rate of success of an individual plant in parenting as many viable embryos as possible (for review see Willson, 1983). Important factors include pollen quantity and quality, quantitative cytological monitoring of pollen germination and tube growth rates in the style, and success of fertilization.

1. *Pollen quantity and quality*

Pollen quantity in mimosoids with polyads is dependent on the number of loculi in each anther. Many genera, including *Acacia*, have strictly eight loculi per anther. Consequently, the polymor-

phism in polyad grain number (see Table 2) results in differences in pollen output per anther (Kenrick & Knox, 1982). Sixteen-grain polyads result in a total pollen production in each anther of 128 grains. Eight-grain polyads result in half this pollen production (i.e., 64 grains) while tetrads result in one-quarter (i.e., 32 grains). Data in Table 1 show that despite these restrictions, the numbers of polyads per flower head are remarkably uniform in some species of *Acacia*.

Pollen viability is particularly important when studying reproduction in *Acacia* because of the existence of a one-to-one polyad–stigma relationship, for example, in *Acacia* (Table 2; Knox & Kenrick, 1983) and in other mimosoid genera (see Table 3). There are various microscopic methods for estimating pollen quality involving different stains such as cotton blue, Alexander's stain (Alexander, 1969), and toluidine blue. These stains all show the presence or absence of cytoplasm (i.e., pollen sterility) only and do not estimate pollen viability, giving positive results long after pollen has ceased to be viable. However, the stains are useful in giving rough estimates of potential fertility, particularly in hybrid progeny where there are often sterile grains which contain little or no cytoplasm. Cotton blue staining combined with separating the individual grains of the polyad has been used to estimate pollen viability in hybrids of *A. nilotica* (Ali & Qaiser, 1980). We have found the fluorochromatic reaction or FCR test (Heslop-Harrison et al., 1984), which relies on enzyme reactions and membrane integrity, to be satisfactory in *Acacia*.

Scoring the compound pollen grains of *Acacia* can be difficult and time-consuming because the central grains overlie one another. One solution is to classify polyads into three classes (Kenrick & Knox, 1985): no grains viable, half or less than half viable, and more than half grains viable (Table 5). This method has the advantage of giving a reasonably quick method of scoring and also of drawing attention to the genetic implications of inviable haploid cells. It is easy to determine if there is a consistent trend in pollen quality in a particular plant because one can see the tetrad arrangement within the polyad. With one exception (Tree 34, Table 5) results compare within 6% of estimates of pollen viability based on *in vivo* pollen tube counts from the same individual (Table 5, B; Kenrick & Knox, 1985).

Acacia pollen varies considerably in viability (Kenrick & Knox, 1985). Once pollen is removed from the anthers, its viability is probably of short duration. In *Acacia retinodes*, quality is typically 70%, but varies widely by more than 90% (Kenrick & Knox, 1985). Pollen quality fell from 49% in polyads with more than 8 grains viable to 9% with the heat treatment (unpublished data). Loss of pollen quality may result from aging of flowers, environmental effects (both current and during development), and the genetic consequences of hybridity (Guinet, 1982; Leach & Whiffen, 1978; Ali & Qaiser, 1980).

2. *Monitoring pollen tube growth*

The cytology of the early stages of fertilization has been followed for only one species of *Acacia* using modern fluorescence techniques (Martin, 1959). More information needs to be obtained concerning pollen tube growth in the style of mimosoids other than *Acacia*. Pollen tubes would have been hard to trace cytologically in the style before the development of fluorescence microscopy (cf. Newman, 1933). The narrow, heavily cutinized style of *Acacia retinodes* presented difficulties in applying the standard decolorized aniline blue fluorescence method for quantitative detection of pollen tubes *in vivo* (Kenrick & Knox, 1985). This is probably because the dye is unable to penetrate the cuticle to bind to the pollen tube walls. However, satisfactory results were eventually obtained by using the technique of D'Souza (1972). This provides simultaneous fixation and clearing in lactic acid–ethanol.

In *A. retinodes*, following compatible cross-pollinations, pollen tubes emerged in a ropelike formation from beneath the polyad and penetrated the stigma. They could not generally be visualized individually until they passed into the transmitting tissue of the style (Kenrick & Knox,

TABLE 5. Estimation of pollen quality in *Acacia retinodes:* **A** by the fluorochromatic reaction (FCR) test; data are mean number of fluorescent grains/polyad ±SE. **B** by comparison of FCR data and cytological monitoring of pollen tube growth. In **B**, only those polyads judged as viable (i.e., containing fluorescent grains by FCR test) have been included in analysis in (i), since in (ii) sterile polyads do not adhere to the stigma and are lost during cytological processing (from Kenrick & Knox, 1985).

	% Polyads with FCR-positive grains		
A Tree	0	1–8	9–16
1	43.2 ± 6.5	43.3 ± 4.3	12.0 ± 3.0
2	6.5 ± 1.2	22.5 ± 3.0	71.2 ± 3.4
3	17.0 ± 6.4	29.3 ± 6.3	54.3 ± 11.4
6	38.0 ± 5.8	35.5 ± 5.3	28.5 ± 9.4
23	6.0 ± 1.7	21.2 ± 2.8	74.0 ± 4.6
34	21.5 ± 5.4	26.8 ± 1.5	52.0 ± 5.6

B Tree	Polyad quality			
	(i) FCR test		(ii) Cytological analysis of pollinated pistils	
	Number of polyads scored	% of total number of polyads with 9–16 positive grains	Number of polyads (pistils)	% polyads with 9–16 tubes in style
1	344	21	132 (129)	15
3	502	62	76 (71)	67
6	384	45	99 (94)	46
23	571	78	29 (28)	72
34	473	66	38 (35)	82
Total	2,274	58	374 (357)	45

1985). Here, each develops a large callose plug (Fig. 8), which makes their identification relatively easy. After passing through the style, the tubes passed over the glandular trichomes in the ovary, to the ovules. Pollen tubes are present in the ovule in fixations made 18 hours after pollination (Kenrick & Knox, 1985). Each pollen tube formed 5–6 callose plugs, which reached greatest intensity in fixations made after tube arrival in the ovule (20 hours or more).

Growth rate of the pollen tubes has been estimated by counting tubes in transects at various distances down the style at different times after pollination. Pollen tubes from some trees grew faster than others (Kenrick & Knox, 1985). There is evidence for biphasic growth, the rate for the first six hours being between 3 and 4.5 $\mu m/min^{-1}$, and from 6 to 11 hours, less than 2 $\mu m/min^{-1}$.

Pollen germination and the entry of the generative cell prior to the vegetative nucleus is reported in *Prosopis spicigera* (Dnyansagar, 1957). Modifications of the standard clearing methods such as those developed by Stelly et al. (1984) have demonstrated that pollen tubes of *Acacia retinodes* pass between cells of the nucellus and enter the embryo sac apparently through the degenerating synergid (Kenrick et al., 1985). Zygotes were visible as early as two days after pollination. Pollen tube entry into the ovules via a synergid is reported in *Neptunia triquetra* (Dnyansagar, 1952).

3. Success of fertilization

In *Acacia retinodes*, fertilization occurs 24–72 hours after pollination; unpollinated flower heads usually undergo abscission between three and seven days after anthesis.

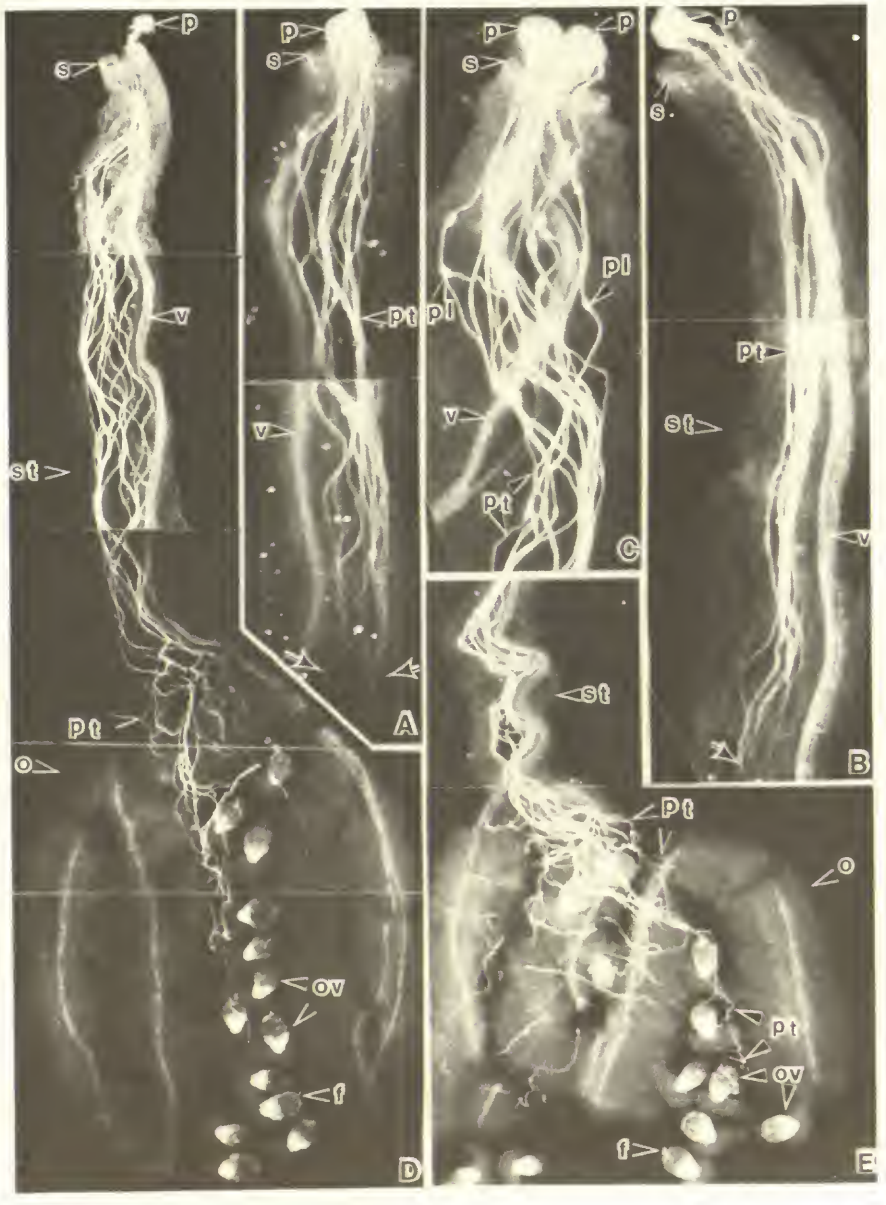

FIGURE 8. Cytology of pollen tube growth in pistils of *Acacia retinodes* following self- and cross-pollination of numbered trees. Epifluorescence illumination of cleared pistils stained with decolorized aniline blue.—A. Cross-pollination 1 × 6 at 6 hrs. Pollen tubes have neared the style base and show only faint fluorescence towards their tips (arrow); callose plugs, although visible, show faint fluorescence.—B. Self-pollination 1 × 1 at 6 hrs. showing features similar to A. Arrow indicates pollen tube tips—C. Cross-pollination 1 × 3 at 11 hrs. showing two polyads on the stigma. Note intense fluorescence of tubes and callose plugs.—D. Cross-pollination 1 × 6 at 48 hrs. showing pollen tubes in ovary and entering ovules (f).—E. Self-pollination 1 × 1 at 48 hrs. showing pollen tubes in ovary and entering ovules (f). The stigma had been pollinated by two polyads, as in C. Magnifications: A, B, C ×200; E, D ×60 (from Kenrick & Knox, 1985).

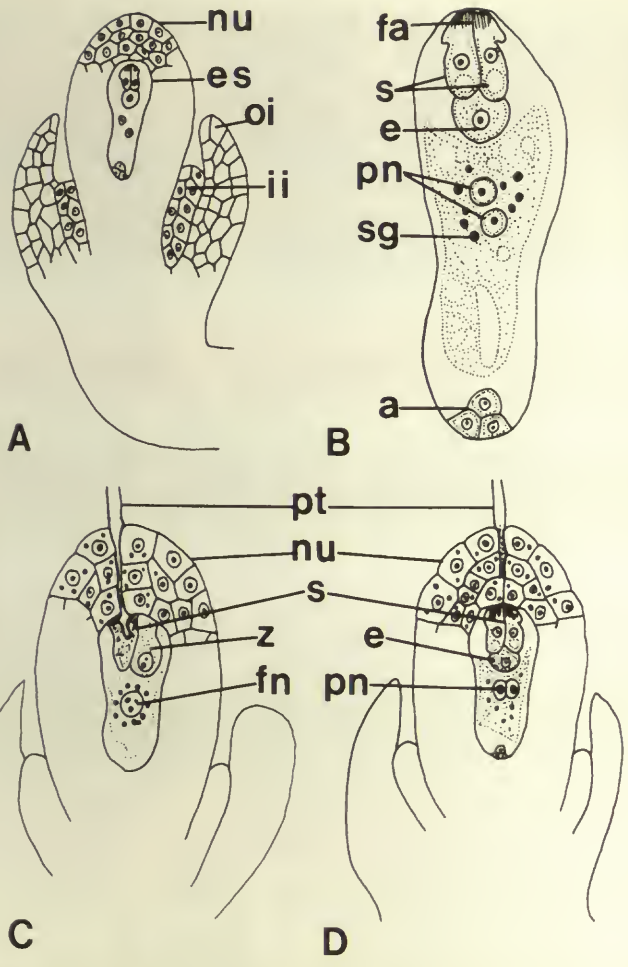

FIGURE 9. Diagrams of ovule and embryo sacs of *Acacia retinodes* based on serial sections (Kenrick et al., 1986).—A. Unfertilized ovule at anthesis.—B. Unfertilized embryo sac showing two synergids with prominent filiform apparatus and large egg.—C. Compatibly cross-pollinated and fertilized ovule showing a pollen tube that has passed between the nucellar cells and entered the embryo sac through one (degenerated) synergid.—D. Self-pollinated, unfertilized ovule in which the pollen tube has been arrested in the nucellus. a, antipodal cells; e, eggs; es, embryo sac; fa, filiform apparatus; fn, triple fusion nucleus; ii, inner integument; nu, nucellus; oi, outer integument; pa, polar nuclei; pt, pollen tube; s, synergid; sg, starch grains; z, zygote.

Fertilized and unfertilized embryo sacs of *A. retinodes* differed in the appearance of key structures (Fig. 9):

In one of the two synergids, the filiform apparatus and its associated nucleus and cytoplasm break down during pollen tube entry.

In the zygote both the cell and nucleus are larger than the unfertilized egg.

In the central cell, after fertilization there is one large fusion nucleus which may have one to three nucleoli present; by contrast, in the unfertilized embryo sac, two much smaller polar nuclei are present (Kenrick et al., 1985).

We can conclude that while the early phases of fertilization are now understood in at least one species of *Acacia,* the process itself and the early stages of embryo development need to be investigated in other mimosoids. *Acacia* is also unusual among angiosperms in that its ovules do not possess a micropyle at the time of fertilization.

4. Self-incompatibility

Self-incompatibility is the inability of a plant to set seed when pollinated with viable pollen from its own flowers. Control is considered to reside with the S (self-incompatibility) gene and its multiple alleles (see de Nettancourt, 1977). The two well-established, genetically inherited self-incompatibility systems which have been documented are, first, the sporophytic system which involves interaction between parentally specified S-alleles of the pollen and the S-alleles of the pistil, found in Compositae and Cruciferae; and second, the gametophytic system, in which the interaction takes place between the gametophytically specified S-alleles of the pollen and the S-alleles of the pistil. This system has been found in the Leguminosae, in *Trifolium* and *Lotus,* where its inheritance is due to a single gene with multiple alleles. Self-incompatibility can be influenced by modifying background genes (Atwood, 1942); by an allele for compatibility (Williams & Williams, 1947); or bypassed by bud pollination and various manipulations of pollen–pistil interactions, including injection of pollen into the ovary cavity, mentor pollen, and environmental temperature effects. Systems of control other than the S-gene have been postulated in gymnosperms (Koski, 1972). There is controversy concerning the genetic control of self-incompatibility (Mulcahy & Mulcahy, 1983, 1985; Lawrence et al., 1985).

Self-incompatibility has been reported in a large number of mimosoid genera (Arroyo, 1981), but in only a few cases have the experimental details been described (Table 6; Bernhardt et al., 1984). Despite the simplicity of floral structure, the small size and abundant stamens of mimosoid flowers have militated against successful breeding experiments. Emasculation was attempted unsuccessfully in *Acacia catechu* (Rao, 1954). In *Leucaena glauca,* Hutton & Gray (1959) used 0.1% solution of a sulphonated lauryl alcohol to remove or kill pollen from the anthers or stigma surface.

Philp & Sherry (1946, 1949), by careful timing of pollination, used *Acacia mearnsii* and *A. decurrens* in breeding and hybridization experiments in South Africa. This work was continued by Moffett (1956, 1965a, b) and Moffett & Nixon (1958, 1974), who established that both *A. mearnsii* and *A. decurrens* set less seed per pod by self- than cross-pollination and that self-pollination produced many plants of low viability. They did not quantify the difference in the number of heads that set pods after self- compared to cross-pollination, although they suggested that more pods were set after cross- than after self-pollination. Moffett & Nixon (1974) concluded that both species were partially self-incompatible, probably due to a system of postzygotic lethal genes similar to that found in some gymnosperms.

In order to explore the breeding system of some Australian species of *Acacia,* we developed new methods for pollination. Phyllodes, open flowers, and green buds were trimmed from shoots with predominantly yellow buds (Newman, 1933). the shoots were then enclosed in cellophane (cellulose acetate) bags to prevent contamination of opening flowers. Three to four days after bagging, the bags were removed, male phase flowers were removed for use in pollen collection, and the remaining female phase flowers were pollinated using pollen collected on the inner face of polyvinyl chloride specimen vials (Bernhardt et al., 1984) with either self-pollen (from the same tree) or cross-pollen (from other trees). The crosses were labelled, rebagged, and the number of heads pollinated in each cross recorded. Some bags were left unpollinated to serve as controls. The cellophane bags were removed from all shoots when the flowers were senescent, about four to seven days after pollination. Pods were counted ten days or longer after pollination and at subsequent intervals until the seed was ripe.

Using this method, we obtained data concerning the number of heads pollinated by self- and

TABLE 6. Breeding system of selected species of *Acacia* and other genera of Mimosoideae, extended from Bernhardt et al., 1984.

Species	Interpretation[a]	Number of trees sampled[c]	Numbers of flowers per pollination per tree	Pollination tested[b,c]	Reference
ACACIA					
A. aroma	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
A. constricta	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
A. cornigera	OB	NG	NG	seed set in glasshouse	Janzen, 1974
A. decurrens	OB	14	flowering branches, 8.3/tree	open 71.9%, self 26.8%, 400 ovules/tree	Philip & Sherry, 1946; Moffett & Nixon, 1974
A. drepanolobium	SI	42	NG	seed set in glasshouse	Hocking, 1970
A. furcatispina	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
A. greggii	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
A. harpophylla	OB	17	NG	number of pods/ cluster, cross 0.47%, self 0.19%–0.26%	Coaldrake, 1977
A. macracantha	SI	6–7	3,786–15,780	cross 0.19%, self 0%, control 0%	Zapata & Arroyo, 1978
A. mearnsii (syn. *A. mollissima*)	OB SI	24 5	flowering branches	number of viable seeds/ pods, open 6.8%, self 2.7%	Moffett, 1956
OTHER MIMOSOIDEAE					
Calliandra laxa	SI	5–6	136–139	cross 11.76%, self 0%, control 0%	Zapata & Arroyo, 1978
C. eriophylla	SI	> 1	1 flowering branch	NG	Simpson, 1977
Enterolobium cyclocarpum	SI	3–5	15–100	number of flowers setting seed, cross 0.28%, self 0%	Bawa, 1974
Inga brenesii	SI	11	205 319	cross 0.35% self 0.01%	Koptur, 1984
I. densiflora	SI	2	79	cross 0.25%, self 0.01%	Koptur, 1984
I. mortoniana	SI	2	53	cross 0.49%	Koptur, 1984
I. oerstediana	SI	3	33 67	self 0.03% cross 0.12%	Koptur, 1984
I. punctata	SI	4	65 146 130	self 0.02% cross 0.20% self 0.02%	Koptur, 1984

TABLE 6. Continued.

Species	Interpretation[a]	Number of trees sampled	Numbers of flowers per pollination per tree[c]	Pollination tested[b,c]	Reference
Leucaena trichodes	SI	NG	NG	NG	Hutton & Eddie, 1982
L. esculenta	SI	NG	NG	NG	Hutton & Eddie, 1982
L. collinsii	SI	NG	NG	NG	Brewbaker, 1982
L. lanceolata	SI	NG	15	NG	Brewbaker, 1982
L. macrophylla	SI	NG	4	NG	Brewbaker, 1982
L. pulverulenta	SI	NG	18	NG	Brewbaker, 1982
L. shannoni	SI	NG	15	NG	Brewbaker, 1982
Pentaclethra macroloba	SI	6	> 180	cross 36%, self 0%	Bawa et al., 1985
Pithecellobium gigantifolium	SI	4	164 396	cross 0.15% self 0%	Bawa et al., 1985
P. saman	SI	3–5	15–100	cross 0.28%, self 0%	Bawa, 1974
Prosopis chilensis	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
P. flexuosa	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
P. velutina	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977
P. torquata	SI	> 1	1 flowering branch	cross, self, control	Simpson, 1977

[a]SI = self-incompatible; OB = outbreeding.
[b]Control pollinations: inflorescences bagged but unmanipulated.
[c]NG = not given.

cross-pollination and the yield of pods from those pollinations (Bernhardt et al., 1984; Kenrick & Knox, 1989). The unmanipulated controls (controlled pollinations), which always include a much greater number of trees than those actually used in the breeding experiments, give a measure of the extent, if any, of self-pollination in the population. They also serve to highlight any effects of floral structure and arrangement, such as that found in *A. myrtifolia* (see Section III. B. 2).

The data obtained from such experimental pollinations demonstrate that *A. retinodes* is highly self-incompatible. Cross-pollinations with other trees resulted in high levels of pod set, while self-pollinations gave few or no pods. Higher levels of pod set resulted from interpopulation crosses than intrapopulation crosses. The self-sterility is almost complete. Controlled self-pollinations, kept in bags during the flowering period to exclude pollinators, show approximately 10% of the pod set of the manipulated self-pollinations. These data indicate that for effective seed set, pollen requires to be transferred from one tree to another. Trees existing very close together, suggesting a common origin from a single parent by root coppicing, were tested for the presence of self-incompatibility. In one such system the individuals were reciprocally cross-incompatible.

Natural pollination results in significantly lower levels of pod set ($P = 0.001$) than manipulated pollination of *Acacia retinodes*. Six pods per infructescence were the greatest number set after open pollination, compared with thirteen after controlled pollination on the same tree. Observations of the presence of polyads on random samples of open-pollinated pistils from six different trees showed that there is considerable variation (Table 7), with pollination ratios varying from 0.1 through 0.9 and a mean ratio of 0.37 (Knox & Kenrick, 1983).

TABLE 7. Frequency of natural pollination of *Acacia retinodes* at Cape Schanck, Victoria (from Kenrick & Knox, 1985).

Tree number	% of pistils pollinated	Number of pistils scored
Population 1:		
1	60	90
3	7	114
6	31	96
Population 2:		
20	54	37
23	24	89
34	88	52
Total:	$x = 37$	478

Trees in natural populations of *Acacia retinodes* show high levels of self-incompatibility. As almost all self-pollinated flowers undergo abscission within seven days of pollination, little self seed is set (Bernhardt et al., 1984; Kenrick & Knox, 1985). Fluorescence microscopy of pistils from trees in these populations showed that after both self- and cross-pollination, pollen tubes germinated, penetrated the stigma, and traveled through the style to the ovary, where they became attached to the micropylar region of the nucellus of ovules. Pollen tubes could not be followed into the tissue of the ovule with fluorescence microscopy. Pollen tube arrest after self-pollination could not be detected prior to the nucellus. The question arises whether the pollen tubes are arrested in the nucellus or in the embryo sac?

We attempted to answer this question in *A. retinodes* by using light microscopy of sectioned or cleared whole ovules. In semithin sections of unpollinated mature ovules, there are three or four layers of tightly packed nucellar cells surrounding the embryo sac (Fig. 9A). Unfertilized embryo sacs 72 hours after anthesis show two synergids with filiform apparatus, an egg cell, a central cell with two polar nuclei, and antipodal cells (Fig. 9B). In fertilized ovules observed 72 hours after pollination, one synergid has degenerated, the zygote has formed, and the polar nuclei have fused with one sperm to form the large triploid fusion nucleus (Fig. 9C). Only rarely in sectioned material is the pollen tube visible. However, in whole ovules cleared by the technique of Stelly et al. (1984), compatible pollen tubes can be traced between the cells of the nucellus into a synergid. At 72 hours after self-pollination, pollen tubes can be seen entering the nucellar tissue but only passing between the first or second layer of cells without any sign of synergid penetration or degeneration (Fig. 9D). These data indicate that the site of pollen tube arrest is in the diploid maternal tissue of the nucellus (Kenrick et al., 1985, 1986).

There is little doubt that self-incompatibility is an important outbreeding mechanism in *Acacia* (see Knox & Kenrick, 1983; Bernhardt et al., 1984) and in other mimosoid legumes (Table 6; Arroyo, 1981). In some Australian species of *Acacia*, including *A. retinodes*, trees are highly self-incompatible. In contrast, other species, notably *A. decurrens* and *A. mearnsii*, show moderate levels of selfing, although seed set is depressed when compared to cross-pollination. In these species, selfed seed is of reduced viability and seedlings are of poor vigor, often with chlorophyll defects (Moffett & Nixon, 1974). The overall conclusion is that *Acacia* is a highly heterozygous outbreeding tree species.

Because of the high levels of self-incompatibility observed in *Acacia retinodes*, we have previously proposed gametophytic control of self-incompatibility (Knox & Kenrick, 1983). The analysis of *Acacia* breeding systems is time-consuming and a long-term exercise, as the product of at least one generation of progeny is involved. In trees that take more than three years from seed to flower production, progeny analysis is rarely a practical proposition. Therefore, we have used the Index of Self-Incompatibility (ISI) introduced by Lloyd (1965) and extended by Zapata & Arroyo (1978).

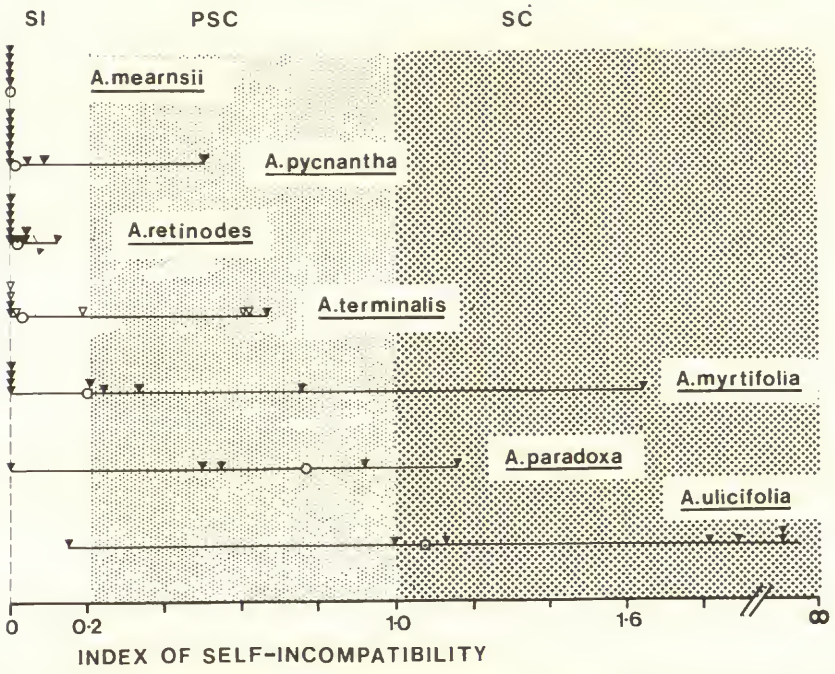

FIGURE 10. Index of self-incompatibility (ISI) for seven species of the nitrogen-fixing tree legume *Acacia*. The index is estimated from the formula: fruit set following self pollination/fruit set following compatible cross-pollination. The horizontal bars show the range of ISI values for each species, the mean is indicated by the open circle, and the arrows indicate individual values. ISI = 0–0.2 self-incompatible (SI); 0.2–1 partially self-incompatible (PSC); ⩾ 1 self-compatible (SC).

This index provides a quantitative estimate of the frequency of self fruit set (or seed set) compared with that following cross-pollination for individual trees (Kenrick & Knox, 1989). Plants were found to vary inter- and intraspecifically (Fig. 10). *Acacia mearnsii*, *A. pycnantha*, and *A. retinodes* have ISI values lower than 0.2 and are considered self-incompatible. *Acacia myrtifolia*, *A. paradoxa*, and *A. terminalis* have ISI values within a wide range, mostly between 0.2 and 0.9, and are considered partially self-compatible. The cultivar *A. ulicifolia* had a mean ISI approaching 1 and so is self-compatible. A model for self-incompatibility in *Acacia* implicates a gametophytic S-gene system overlying a recessive postzygotic lethal or sublethal gene system (Kenrick & Knox, 1989). The latter system is expressed in situations where an Sc (compatible) allele is present.

V. Conclusions and Future Perspectives

The frontiers of knowledge of pollen–pistil interactions are reached in our understanding of one genus of mimosoids, namely *Acacia*. The techniques applied to this genus now need to be extended to other mimosoids, to see how widely applicable the concepts really are. A range of variation in reproductive strategies is likely to occur in a group as large as the Mimosoideae, and so generalizations from one very large genus are not necessarily typical of the whole. The present state of knowledge of *Acacia* pollen–pistil interactions may be summarized in terms of four main areas of research.

Floral biology. Despite the remarkable homology of the flower head in gross morphology in mimosoids, detailed analysis in *Acacia* showed that there is wide variation between species in flower number, anther number, and even style length. Further work is needed to relate these characters with pollen–stigma interactions, pollinator preferences (see Bernhardt, this volume), and fecundity in these melittophilous flower heads. *Acacia* flower heads are protogynous, with the female and male phases separated temporally. This dichogamy encourages outbreeding, and is associated with various devices to attract pollinators, including scent production from osmophores in the anther walls at male phase, floral nectaries, and anther glands. A remarkable feature of *Acacia* among mimosoids is the apparent link in the genetic mechanism correlating the grains in the polyad, and ovule number per ovary. This mechanism profoundly affects fecundity because of the rigid control of polyad output per anther. Some species avoid this by increasing anther number per flower or flower head.

Pollen biology. Differentiation of the generative cell in the bicellular pollen occurs relatively late in development in *Acacia*. Maturation in terms of elongation of the cell into a central site and the shape change associated with dormancy occurs in the final 24 hours before dehiscence, prior to male phase of flowering. At the same time unusual membrane labyrinths containing masses of coated vesicles are differentiated at each aperture. These are implicated in transport of proteins to the surface at germination or in early processes of pollen tube growth.

Pistil biology and pollination. The stigma of *Acacia* is very small—60 μm diam. in *A. retinodes,* set on a style about 1.5 mm long. The saucer-shaped stigma cells bear a copious exudate when receptive. This probably functions to trap the polyads from the surface of passing insects. The differentiation patterns and tentative chemical composition have been explored by cytochemical and ultrastructural studies. The most remarkable feature of this small stigma is that it appears to function as a nectary gland, secreting a droplet of fluid following pollination. This secretion functions either as a germination medium or through surface tension effects to increase the probability of a 1:1 polyad–stigma relationship. Secretion of the exudate occurs within 30 minutes of pollination and is pollen-requiring, triggered apparently by surface aqueous or alcohol soluble fractions.

Fertilization. There is no stigma surface cuticle, and pollen tubes penetrate in ropelike formation into the style. This is of the solid type, and tubes pass through the intercellular spaces of the transmitting tissue, reaching the ovary within 18 hours of pollination. There is cytological evidence for biphasic tube growth, initially 4.5 μm/min in the stigma and upper style, later reduced to less than half this rate in the lower style and ovary. Pollen tubes enter the ovules by passing through the cell walls of the nucellus and entering a synergid to effect double fertilization.

Self-incompatibility. Many species of mimosoids are self-incompatible, and in *Acacia* genetic experiments suggest that this is controlled by a gametophytic *S*-gene system. However, the responses of individual trees, which can show different levels of compatibility, has led to a new model. Self-incompatibility is considered to be controlled by a *S*-gene system overlying a system of lethal or sublethal genes which are expressed when a *Sc* (compatible) allele is present. Self-incompatibility is important as an outbreeding mechanism, underlining the effectiveness of pollinating insects and vertebrates in increasing fecundity.

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References

Adams, C. D. 1972. Flowering Plants of Jamaica. University of the West Indies, Mona Jamaica.

Alexander, M. P. 1969. Differential staining of aborted and non-aborted pollen. Stain Technol. 44: 117–122.

Ali, S. I. and Qaiser, J. 1980. Hybridization in *A. nilotica* (Mimosoideae) complex. Bot. J. Linn. Soc. 80: 69–77.

Armitage, I. 1977. Acacias of New South Wales. Society for Growing Australian Plants, Sydney.

Arroyo, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Atwood, S. S. 1942. Genetics of pseudo-self-compatibility and its relation to cross-compatibility in *Trifolium repens*. J. Agric. Res. 64: 699.

Barlow, B. A. 1981. The Australian flora: its origin and evolution. Pp. 25–75 *in* A. E. George (editor), Flora of Australia, Vol. 1. Australian Government Publishing Service, Canberra.

Barth, O. M. 1965. Feinstruktur des sporoderms einiger Brasilianischer Mimosoiden–Polyaden. Pollen & Spores 7: 429–441.

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85–92.

_____, D. R. Perry & J. H. Beach. 1985. Reproductive biology of tropical lowland rainforest trees 1. Sexual systems and incompatibility mechanisms. Amer. J. Bot. 72: 331–345.

Bernhardt, P., J. Kenrick & R. B. Knox. 1984. Pollination biology and the breeding system of *Acacia retinodes* (Leguminosae: Mimosoideae). Ann. Missouri Bot. Gard. 71: 17–29.

Brewbaker, J. L. 1982. Systematics, self-incompatibility breeding systems and genetic improvement of *Leucaena* species. Leucaena 1982 Workshop, Singapore, Nov. 1982.

Buttrose, M. S., W. J. R. Grant & M. Sedgley. 1981. Floral development in *Acacia pycnantha* Benth. in Hook. Austral. J. Bot. 29: 385–395.

Carr, J. D. 1976. South African Acacias. Conservation Press, Johannesburg.

Coaldrake, J. E. 1971. Variation in floral, seed and growth characteristics of *Acacia harpophylla* (Brigalow). Austral. J. Bot. 19: 335–352.

Coetzee, J. A. 1955. The morphology of *Acacia* pollen. S. African J. Sci. 52: 23–27.

Cookson, I. C. 1954. The Cainozoic occurrence of *Acacia* in Australia. Austral. J. Bot. 2: 52–59.

Davies, S. J. J. F. 1976. Studies of the flowering season and fruit production of some arid zone shrubs and trees in Western Australia. J. Ecol. 64: 665–687.

Derstine, K. S. & S. C. Tucker. 1985. Histological floral ontogeny of *Acacia baileyana* (Leguminosae: Acacia). Amer. J. Bot. 72: 812–813.

Dnyansagar, V. R. 1952. Embryological studies in the Leguminosae. IV. A contribution to the embryology of *Neptunia triguetra* Benth. Proc. Indian Acad. Sci. 36: 1–11.

_____. 1954. Embryological studies in leguminosae. VI. Inflorescence, Sporogenesis and gametophytes of *Dichiostacys cinerea* W. & A. and *Parkia biglandulosa* W. & A. Lloydia 17: 263–274.

_____. 1955. Embryological studies in the leguminosae. XI. Embryological features and formula and taxonomy of the Mimosaceae. J. Indian Bot. Soc. 34: 362–374.

_____. 1957. Embryological studies in the leguminosae. V. *Prosopis spicigera* and *Desmanthus virgatus*. Bot. Gaz. (Crawfordsville) 118: 180–186.

_____. 1958. Embryological studies in the leguminosae. VIII. *Acacia auriculaeformis* A. Cunn., *Adenanthera pavonia* Linn., *Calliandra lematocephala* Hassk. and *Calliandra grandiflora* Benth. Lloydia 21: 1–25.

D'Souza, L. 1972. Staining pollen tubes in the styles of cereals with cotton blue: fixation by ethanol-lactic acid for enhanced differentiation. Stain Technol. 47: 107–108.

Elias, T. S. 1974. The genera of Mimosoideae (Leguminosae) in south-eastern United States. J. Arnold Arbor. 55: 67–118.

_____. 1981. Mimosoideae. Pp. 143–151 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Ganeshaiah, K. N., R. Uma Shaanker & G. Shivashankar. 1986. Stigmatic inhibition of pollen grain germination—its implications for frequency distribution of seed number in pods of *Leucaena leucephala* (Lam) de Wit. Oecologia 70: 568–572.

Geitler, L. 1927. Zur morphologie du Inflorezenzen und Bluten von *Neptunia oleraceae* Bor. Ber. Deutsch. Bot. Ges. 45: 36–39.

Gordon-Gray, K. D. & C. J. Ward. 1975. A contribution to the knowledge of floral variation in *Acacia karroo* in eastern South Africa. Biossiera 24: 279–281.

Guinet, P. 1969. Les Mimosacées: étude de palynologie fondamentale, correlations, evolution. *In* Travaux de la Section Scientifique et Technique, Vol. 9. Le Mission Pondichery.

_____. 1981. Mimosoideae: the characters of the pollen grains. Pp. 835–857 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____. 1982. Distribution of the pollen characters in some hybrids and in some suspected hybrids in the genus *Acacia*. Bull. Int. Group for the Study of Mimosoideae 11: 25–28.

_____. 1986. Geographic patterns of the main pollen characters in genus *Acacia* Leguminosae with particular reference to subgenus *Phyllodineae*. Pp. 297–311 *in* S. Blackmore & K. Ferguson (editors), Pollen & Spores: Form and Function. Academic Press, London.

_____ & B. Lugardon. 1976. Diversité des structures de l'exine dans le genre *Acacia* (Mimosaceae). Pollen & Spores 18: 483–511.

Heslop-Harrison, J. & Y. Heslop-Harrison. 1982. Pollen stigma interactions in the Leguminosae: constituents of the stylar fluid and stigma secretion of *Trifolium pratense* L. Ann. Bot. (London) 49: 729–735.

_____, _____ & K. R. Shivanna. 1984. The evaluation of pollen quality and further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67: 367–375.

Heslop-Harrison, Y. 1981. Stigma characteristics and angiosperm taxonomy. Nordic J. Bot. 1: 401–420.

_____ & J. Heslop-Harrison. 1983. Pollen stigma interaction in the Leguminosae. The secretory system of the style in *Trifolium pratense* cultivar Saltoron. Ann. Bot. (London) 50: 635–646.

Hocking, B. 1970. Insect associations with the swollen-thorn Acacias. Trans. Roy. Entomol. Soc. London. 122: 211–255.

Hopkins, H. C. 1983. Taxonomy, reproduction biology and economic potential of *Parkia* Leguminosae, Mimosoideae in Africa and Madagascar. Bot. J. Linn. Soc. 87: 135–168.

_____. 1984. Floral biology and pollination and ecology of the neotropical species of *Parkia*. J. Ecol. 72: 1–23.

_____. 1986. *Parkia* (Leguminosae: Mimosoideae). Fl. Neotrop. Monogr. 43.

Hutton, E. M. & T. Z. Eddie. 1982. *Leucaena esculenta* and *L. trichodes*—some similarities and differences. *Leucaena* Res. Rep. 3: 12–13.

_____& S. C. Gray. 1959. Problems in adapting *Leucaena glauca* as a forage for the Australian tropics. Empire J. Exp. Agric. 27: 187–196.

Janzen, D. H. 1974. Swollen-thorn *Acacias* of Central America. Smithsonian Contr. Bot. 13.

Jepson, W. L. 1951. A Manual of the Flowering Plants of California. Univ. California Press. Berkeley & Los Angeles.

Kenrick, J. & R. B. Knox. 1979. Pollen development and cytochemistry in some Australian species of *Acacia*. Austral. J. Bot. 27: 413–427.

_____ & _____. 1981a. Structure and histochemistry of the stigma and style of some Australian species of *Acacia*. Austral. J. Bot. 29: 733–745.

_____ & _____. 1981b. Post-pollination exudate from stigmas of *Acacia* (Mimosaceae). Ann. Bot. (London) 48: 103–106.

_____ & _____. 1982. Function of the polyad in reproduction of *Acacia*. Ann. Bot. (London) 50: 721–727.

_____ & _____. 1985. Self-incompatibility in the nitrogen-fixing tree, *Acacia retinodes:* quantitative cytology of pollen tube growth. Theor. Appl. Genet. 69: 481–488.

_____ & _____. 1989. Quantitative analysis of self-incompatibility in trees of seven species of *Acacia*. J. Heredity 80: 240–245.

_____, V. Kaul & E. G. Williams. 1986. Self incompatibility in *Acacia retinodes:* site of pollen tube arrest is the nucellus. Planta 169: 245–250.

_____, _____, R. B. Knox & E. G. Williams. 1985. The nucellus: site of self-incompatible pollen tube arrest in *Acacia retinodes*. Pl. Self Incompatibility Newslett. 17: 17–19.

_____, P. Bernhardt, R. Marginson, G. Beresford, R. B. Knox, I. Baker & H. G. Baker. 1987. Pollination-related characteristics in the mimosoid legume *Acacia terminalis*. Pl. Syst. Evol. 157: 49–62.

Knox, R. B. & J. Kenrick. 1983. Polyad function in relation to the breeding system of *Acacia*. Pp. 411–417 *in* D. Mulcahy & E. Ottaviano (editors), Pollen Biology. Elsevier-North Holland.

_____ & C. McConchie. 1986. Structure and function of compound pollen. Pp. 265–282. *in* S. Blackmore & K. Ferguson (editors), Pollen and Spores: Form and Function. Academic Press, London.

_____, J. Kenrick, S. Jobson & C. Dumas. 1989. Reproductive function in the mimosoid legume *Acacia retinodes*: Ultrastructure and cytochemical characteristics of stigma receptivity. Austral. J. Bot. 37: 103–124.

_____, _____, _____, P. Bernhardt, R. Marginson, G. Beresford, I. Baker & H. G. Baker. 1985. Extra-floral nectaries as adaptations for bird pollination in *Acacia terminalis*. Amer. J. Bot. 72: 1185–1196.

KOPTUR, S. 1984. Outcrossing and pollinator limitation of fruit set: breeding systems of neotropical *Inga* trees (Fabaceae: Mimosoideae). Evolution 38: 1130–1143.

KOSKI, V. 1972. On self-pollination, genetic load and subsequent inbreeding in some conifers. Commun. Inst. Fenniae 78: 5–40.

KREITNER, G. L. & E. L. SORENSEN. 1984. Stigma development and the stigmatic cuticle of alfalfa. *Medicago sativa* L. Bot. Gaz. (Crawfordsville) 145: 436–443.

LATTING, J. 1961. The biology of *Desmanthus illinoensis*. Ecology 42: 487–493.

LAWRENCE, M. J., D. F. MARSHALL, V. E. CURTIS & C. H. FEARON. 1985. Gametophytic self-incompatibility re-examined: a reply. Heredity 54: 131–138.

LEACH, G. J. & T. WHIFFEN. 1978. Analysis of a hybrid swarm between *Acacia brachybotrya* and *A. calamifolia* (Leguminosae). Bot. J. Linn. Soc. 76: 53–69.

LEWIS, G. P. & T. S. ELIAS. 1981. Tribe 3. Mimoseae Bronn. (1822). Pp. 155–168 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

LLOYD, D. G. 1965. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contr. Gray Herb. 195: 3–134.

MCCOY, K. & R. B. KNOX. 1988. The plasma membrane and generative cell organization in pollen of the mimosoid legume, *Acacia retinodes*. Protoplasma 143: 85–92.

MAHESHWARI, P. 1931. Contributions to the morphology of *Albizzia lebbek*. J. Indian Bot. Soc. 10: 241–264.

MARGINSON, R., M. SEDGLEY & R. B. KNOX. 1985. Physiology of post-pollination exudate production in *Acacia*. J. Exp. Bot. 36: 1660–1668.

MARTIN, F. W. 1959. Staining and observing pollen tubes by means of fluorescence. Stain Technol. 34: 125–128.

MILTON, S. J. & E. J. MOLL. 1982. Phenology of Australian *Acacias* in the S.W. Cape, South Africa, and its management implications for management. Bot. J. Linn. Soc. 84: 295–327.

MOFFETT, A. A. 1956. Genetical studies in *Acacia*. I. The estimation of natural crossing black wattle. Heredity 10: 57–67.

_____. 1965a. Genetical studies in *Acacia*. III. Chlorosis in interspecific hybrids. Heredity 20: 609–620.

_____. 1965b. Genetical studies in *Acacia*. IV. Natural hybrids between *Acacia irrorata* and *A. mearnsii*. Heredity 20: 621–629.

_____ & K. M. NIXON. 1958. Genetical studies in *Acacia*. II. Leaf characters in hybrids between green and black wattles. Heredity 12: 199–212.

_____ & _____. 1974. The effects of self-fertilization on green wattle (*Acacia decurrens* (J. Wendl.) Willd.) and black wattle (*A. mearnsii* de Wild.). Wattle Res. Inst. Rep. 1973–74.

MORRISON, D. A. 1986. A Demographic Analysis of Reproduction in *Acacia suaveolens*. Ph.D. thesis. University of Sydney.

MUELLER, FERDINAND VON. 1887–1888. Inconography of Australian species of *Acacia* and Cognate Genera. Robert S. Brain, Government Printer, Melbourne.

MULCAHY, D. L. & G. B. MULCAHY. 1983. Gametophytic self-incompatibility re-examined. Science 220: 1247–1251.

_____ & _____. 1985. Gametophytic self-incompatibility or the more things change . . . Heredity 54: 139–144.

MURRAY, D. R., W. J. ASHCROFT, R. D. SEPPELT & F. G. LENNOX. 1978. Comparative biochemical and morphological studies in *Acacia sophorae* (Labill.) R. Br. and *A. longifolia* (Andrews) Willd. Austral. J. Bot. 26: 755–771.

NARASIMHACHAR, S. G. 1948. A contribution to the embryology of *Acacia farnesiana* L. (Willd.). Proc. Indian Acad. Sci. 28: 144–149.

NETTANCOURT, D. DE. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin & New York.

NEVLING, L. I. AND T. S. ELIAS. 1971. *Calliandra naematocephala*. History, morphology and taxonomy. J. Arnold. Arbor. 52: 69–85.

NEWMAN, I. V. 1933. Studies in the Australian Acacias. II. The life history of *Acacia baileyana* (FvM) Part 1. Some ecological and vegetative features, spore production and chromosome number. J. Linn. Soc. Bot. 99: 145–171.

_____. 1934a. Studies in the Australian Acacias. III. Supplementary observations on the habit, carpel, spore production and chromosomes of *Acacia baileyana* F.V.M. Proc. Linn. Soc. New South Wales 59: 237–251.

_____. 1934b. Studies in the Australian Acacias. IV. The life history of *Acacia baileyana* F.V.M. Part 2. Gametophytes, fertilization, seed production and germination, and general conclusion. Proc. Linn. Soc. New South Wales 59: 277–313.

_____. 1936. Studies in the Australian Acacias. VI. The meristematic activity of the floral apex of *Acacia longifolia* and *Acacia suaveolens* as a histogenetic study of the ontogeny of the carpel. Proc. Linn. Soc. New South Wales 61: 56–88.

Pedley, L. 1978–1979. A revision of *Acacia* Mill. in Queensland. Austrobaileya 1: 17–315.

Philp, J. & S. P. Sherry. 1946. The degree of natural crossing in green wattle (*A. decurrens* Willd.) and its bearing on wattle breeding. J. S. Af. For. Ass. 14: 1–28.

_____ & _____. 1949. The genetics of hybrids between green wattle (*A. decurrens* Willd.) and black wattle (*A. mollissima*). J. S. African Forest. Assoc. 17: 6–58.

Preece, P. B. 1971. Contributions to the biology of Mulga. I. Flowering. Austral. J. Bot. 19: 21–38.

Rao, H. S. 1954. Pollination mechanism in *Acacia catechu*. J. Indian Bot. Soc. 33: 93–97.

Robbertse, P. J. 1974. The genus *Acacia* in South Africa II, with special reference to the morphology of the flower and inflorescence. Phytomorphology 24: 1–15.

Rock, J. F. 1913. The indigenous trees of the Hawaiian Islands. Published under patronage, Honolulu.

Sargent, C. S. 1922. Manual of the trees of North America (exclusive of Mexico). Houghton Mifflin, Cambridge, The Riverside Press.

_____. 1947. The silva of North America, Volume III. Peter Smith, New York. The Murray Printing Co., Cambridge, Massachusetts.

Sedgley, M. 1985. Some effects of temperature and light on floral initiation and development in *Acacia pycnantha*. Austal. J. Pl. Physiol. 12: 109–118.

Selling, O. H. 1947. Studies in Hawaiian Pollen Statistics. Bishop Museum, Honolulu, Hawaii.

Siegel, S. 1956. Non-parametric Statistics for the Behavioral Sciences. McGraw Hill, Kogakusha.

Simpson, B. B. 1977. Breeding systems of dominant perennial plants of two disjunct warm desert ecosystems. Oecologia 27: 203–226.

Singh, B. & T. N. Shivapuri. 1935. The gametophytes of *Neptunia oleracea* Lour. Proc. Indian Acad. Sci. 1: 423–434.

Sinha, S. C. 1971. Floral morphology of *Acacias*. Caribbean J. Sci. 11: 136–153.

Solbrig, O. T. & P. D. Cantino. 1975. Reproductive adaptations in *Prosopis* (Leguminosae, Mimosoideae). J. Arnold. Arbor. 56: 185–210.

Stelly, D. M., S. J. Peloquin, R. G. Palmer & C. F. Crane. 1984. Mayer's hemalum-methyl salicylate: a stain clearing technique for observations within whole ovules. Stain Technol. 59: 155–161.

Theunis, C. H., C. A. McConchie & R. B. Knox. 1985. Three-dimensional reconstruction of the generative cell and its wall connection in mature bicellular pollen of *Rhododendron*. Micron & Microscopia Acta 16: 225–231.

Tucker, S. C. 1984a. Developmental basis for heteromorphy in *Neptunia plena* (Leguminosae: Mimosoideae). Amer. J. Bot. 71: 835.

_____. 1984b. Unidirectional organ initiation in leguminous flowers. Amer. J. Bot. 71: 1139–1148.

Tuohy, M., C. McConchie & R. B. Knox. 1987. Computer-assisted three dimensional reconstruction technology in plant cell image analysis: applications of interactive computer graphics. J. Microscopy 147: 83–88.

Vishnu-Mittre & B. D. Sharma. 1962. Studies of Indian pollen grains. I. Leguminosae. Pollen & Spores 4: 5–45.

Waha, M. 1984. Zur Ultrastruktur und Funktion pollenverbindender Faden bei Ericaceae und anderen. Angiospermen familien. Pl. Syst. Evol. 147: 189–203.

Waser, N. M. & M. V. Price. 1983. Optimal and actual outcrossing in plants, and the nature of plant–pollinator interaction. Pp. 339–359 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York.

Wiggins, I. L. & D. M. Porter. 1971. Floral of the Galapagos Islands. Stanford Univ. Press, Stanford, California.

Williams, R. D. & W. Williams. 1947. Genetics of red clover (*Trifolium pratense* L.) compatibility II (a) Homozygous self-sterility $S_x S_x$ genotypes obtained as a result of pseudo-fertility; (b) self-fertility. J. Genet. 48: 51–68.

Willson, M. F. 1983. Plant Reproductive Ecology. John Wiley & Sons, Toronto & New York.

Windler, D. R. 1966. A revision of the genus *Neptunia* (Leguminosae). Austral. J. Bot. 14: 379–420.

Wirjodarmodjo, H. & P. Wiroatmodjo. 1983. *Leucaena leucocephala:* the Indonesian experience. FAO. Regional Office for Asia & Pacific, Bangkok.

Wodehouse, L. P. 1935. Pollen Grains: Their Structure, Identification and Significance in Science and Medicine. McGraw Hill, New York.

Zapata, T. R. & M. T. K. Arroyo. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10: 221–230.

POLLEN–PISTIL INTERACTIONS (PAPILIONOIDEAE)

K. R. Shivanna[1] and Simon J. Owens[2]

Abstract

Investigations on the structural organization of the pistil and on pollen–pistil interactions in papilionoid legumes have received serious attention only during the last decade and are limited to only a few taxa. Basically, there are two stigma types. In the first type, the stigma is well-developed, papillate, and produces copious exudate. The exudate is held below the stigma cuticle and is released onto the stigma surface only after the disruption of the cuticle, which may occur during stigma development or during tripping by visiting insects. In the second type, the stigma is distinctly papillate and does not produce exudate or produces only a limited amount confined to the interstices of papillae. The cuticle is not lifted as a continuous stigmatic membrane. The style exhibits characteristics of both solid and hollow styles. The upper part of the stylar cavity is irregular and develops lysigenously during ontogeny due to degeneration of cells in the core of the "solid" transmitting tissue. The lower part of the stylar cavity is regular and is bordered by one or a few layers of glandular canal cells; it does not show any degeneration of cells. The period of receptivity of the stigma is variable. In many species the stigma becomes receptive 1–3 days before anthesis. However, in species in which the exudate is held below the intact cuticle until anthesis, the stigma becomes receptive only after the rupture of cuticle following tripping. Although most of the papilionoid legumes are self-compatible, the extent of self-pollination and fertilization is highly variable. Pollination and fertilization depend on the architecture of the flower, particularly the pistil (which permits or prevents self-pollen coming in contact with the stigma), the nature of the stigmatic membrane, and self-incompatibility. Self-incompatibility, reported in a few cultivated taxa, is gametophytically controlled and is governed by multiple alleles at one locus. Apart from an extension of studies on the structural aspects of the pollen–pistil interaction, both at light and electron microscopic levels, there is considerable scope for experimental studies relevant both to fundamental and applied areas of reproductive biology of papilionoid legumes.

I. Introduction

Although papilionoid legumes have attracted the attention of reproductive biologists for a long time, most of the earlier studies were concerned with pollination biology and breeding systems. These investigations have been reviewed extensively in *Advances in Legume Systematics, Part 2* (see Arroyo, 1981). Some studies have also been conducted on self-compatibility (see Free, 1970). Investigations on the structural details of the pistil and pollen–pistil interaction have received serious attention only during the last decade (Table 1). These studies are largely confined to morphological features of the stigma and style, and anatomical details are available only for a limited number of species. In particular, there is very little information on the pollen–pistil interaction. In this article, the available information is briefly reviewed, and the major gaps in our knowledge are indicated.

II. The Stigma

morphology and anatomy

The stigma is terminal as in *Arachis, Cajanus, Crotalaria,* and *Trifolium* or subterminal as in *Phaseolus* and *Vigna*. The style is bifid in some taxa. In *Lotus berthelotii* (Owens, 1985), the

[1]Department of Botany, University of Delhi, Delhi 110007, India.
[2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K.

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Monogr. Syst. Bot. Missouri Bot. Gard. 29:157–182. 1989.

TABLE 1. Studies on the structural details of the pistil and pollen–pistil interaction in papilionoid legumes. +, data available.

Tribe	Genus	Voucher[1]	Stigma	Style	Pollen–pistil interaction	Sources (Unless otherwise noted, references are personal communication.)
Swartzieae	*Swartzia myrtifolia*	KHSCN 46418		+		Owens
	S. apetala	KHSCN 38281		+		Owens
Sophoreae	*Castanospermum australe*[2]	Prakash & Cameron F 001	+	+		Shivanna
	Cladrastis kentukea[2]	Prakash & Cameron F 007	+	+		Shivanna
	Myroxylon peruiferum	KAN 000-73-12286	+			Owens
	Sophora japonica	KAN 000-72-12999	+			Owens
	S. macrocarpa	KAN 398-73-05027	+			Owens
Dalbergieae	*Dalbergia sissoo*	Shivanna 0013	+	+		Shivanna
Millettieae	*Pongamia glabra*	Shivanna 0014	+	+		Shivanna
	Tephrosia purpurea	Shivanna 0016	+	+		Shivanna
	T. bracetolata	KHSCN 15510		+		Owens
	Wisteria sinensis	KAN 000-73-20755	+			Owens
Indigofereae	*Cyamopsis tetragonoloba*	Shivanna 0017	+	+		Shivanna
	Indigofera spicata	Stirton 8831	+			Owens
Phaseoleae	*Cajanus cajan*		+	+		Ghosh & Shivanna, 1982
	Erythrina variegata	Shivanna 0018	+	+		Shivanna
	E. corallodendron	KAN 148-45-14801	+			Owens
	Glycine max		+	+	+	Tilton et al., 1984
	Hardenbergia violacea[2]	Prakash & Cameron F 008	+	+		Shivanna
	Kennedia stirlingii	KAN 167-82-01357	+			Owens
	Lablab purpureus	Shivanna 0012	+	+		Shivanna
	L. niger	KHSCN 24415		+		Owens
	Phaseolus sp.		+	+	+	Hawkins & Evans, 1973; Webster et al., 1977, 1979; Lord & Webster, 1979; Halterlein et al., 1980; Heslop-Harrison & Heslop-Harrison, 1984; Lord & Kohorn, 1986

TABLE 1. Continued.

Tribe	Genus	Voucher[1]	Stigma	Style	Pollen–pistil interaction	Sources (Unless otherwise noted, references are personal communication.)
	Vandasia retusa[2]	Prakash & Cameron F 002	+	+		Shivanna
	Vigna unguiculate		+	+		Ghosh & Shivanna, 1982
Aeschynomeneae	*Aeschynomene carvalhoi*	KHSCN 49461		+		Owens
	A. katangensis	KHSCN 3214/2		+		Owens
	Amicia zygomeris	KAN 688-65-68801	+			Owens
	Arachis hypogaea		+	+		Lakshmi & Shivanna, 1985
Galegeae	*Astragalus bigelovii* var. *thompsonii*	KAN 479-79-05340	+			Owens
	Caragana fruticosa[2]		+	+		Shivanna
	C. arborescens	KAN 000-69-16387	+			Owens
	Swainsona sp.[2]	Prakash & Cameron F 006	+	+		Shivanna
Loteae	*Lotus berthelotii*	KAN 297-72-06058	+		+	Owens, 1985; Bubar, 1958
Coronilleae	*Coronilla emerus*	KAN 118-68-11823	+			Owens
	Hippocrepis balearia	KAN 050-77-00223	+			Owens
Vicieae	*Pisum* sp.	Shivanna 0011	+	+		Shivanna
	Vicia faba		+		+	Toynbee-Clarke, 1971, 1974; Lord & Heslop-Harrison, 1984
Cicereae	*Cicer arietinum*		+	+	+	Savithri et al., 1980; Malti & Shivanna, 1983; Turano et al., 1983
	C. reticulum	Shivanna 0008	+	+	+	Malti & Shivanna
	C. pinnatifidum	Shivanna 0009	+	+	+	Malti & Shivanna
	C. judaicum	Shivanna 0010	+	+	+	Malti & Shivanna
Trifolieae	*Medicago sativa*		+			Kreitner & Sorensen, 1984, 1985; Small & Brookes, 1983
	Trifolium pratense		+	+	+	Heslop-Harrison & Heslop-Harrison, 1982a, b, 1983

TABLE 1. Continued.

Tribe	Genus	Voucher[1]	Stigma	Style	Pollen–pistil interaction	Sources (Unless otherwise noted, references are personal communication.)
Mirbelieae	*Brachysema lanceolata*	KAN 167-82-01356	+			Owens
	Oxylobium scandens[1]	Prakash & Cameron F 004	+	+		Shivanna
	Pultenea retusa[2]	Prakash & Cameron F 009	+	+		Shivanna
Bossiaeeae	*Hovea lanceolata*[2]	Prakash & Cameron F 005	+	+		Shivanna
Crotalarieae	*Crotalaria retusa*		+	+	+	Malti & Shivanna, 1984
Thermopsideae	*Thermopsis divaricarpa*	KAN 000-73-20755	+			Owens
Genisteae	*Cytisus villosus*	KAN 156-82-01268	+			Owens
	Genista linifolia	KAN 458-67-45803	+			Owens
	Laburnum anagyroides	KAN 393-71-03784	+			Owens
	Lupinus sp.	Shivanna 0015	+	+		Shivanna
	Pettaria sp.[2]	Prakash & Cameron F 003	+	+		Shivanna

[1]KAN-Kew accession number; KHSCN-Kew Herbarium Spirit Collection number, Stirton 000. Specimen collected by Stirton; Shivanna 0008-0018 numbers of specimens; Prakash & Cameron F 001-F 009 numbers of fixed materials, deposited in Delhi University Herbarium (DUH).

[2]Studies confined to fixed material.

shorter arm of the stigma terminates in a heavily cutinized papillate surface, whereas the longer one terminates in the receptive stigmatic surface.

In members of the Sophoreae *(Castanospermum, Cladrastis)*, the stigma is small and is represented by a group of papillae or a mound of glandular cells at the tip of a slender style (Figs. 1–3). The stigma does not have a whorl of nonreceptive hairs at the base. It is wet, but the exudate appears to be sparse. In *Dalbergia, Pongamia, Cyamopsis*, and members of many advanced tribes, particularly Phaseoleae, Vicieae, and Trifolieae, the stigma is capitate or flattened and is clearly demarcated from the style. The stigma produces copious exudate and often bears a whorl of nonreceptive hairs at the base, as in several members of Phaseoleae and Millettieae (Figs. 4–6; Table 4). The surface of the stigma is smooth in *Dalbergia, Erythrina*, and *Lablab* (Shivanna, unpublished) *Cajanus* (Ghosh & Shivanna, 1982), *Trifolium* (Heslop-Harrison & Heslop-Harrison, 1983), *Glycine* (Tilton et al., 1984), *Medicago* (Kreitner & Sorensen, 1984, 1985), and *Phaseolus* (Heslop-Harrison & Heslop-Harrison, 1984; Lord & Kohorn, 1986). In *Melilotus, Trigonella* (Small & Brookes, 1983), and *Vicia* (Lord & Heslop-Harrison, 1984), the stigma surface is covered with unicellular papillae and produces copious exudate.

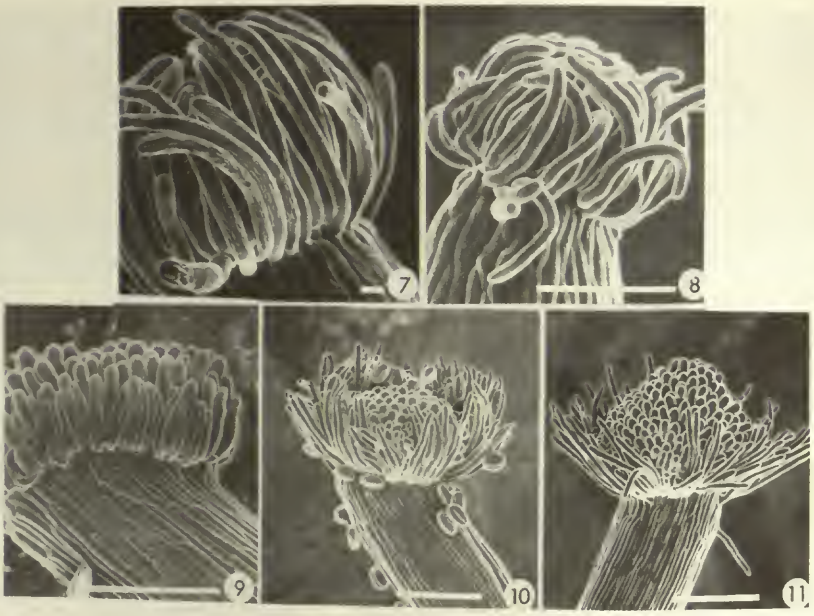
The stigma remains small even in members of some of the advanced tribes (e.g., *Caragana, Arachis, Swainsona, Cicer*, and *Crotalaria*). However, many of these have a whorl of nonreceptive hairs at the base. The stigma in these advanced tribes is distinctly papillate and in most taxa the papillae are unicellular. In *Arachis*, however, the papillae are multicellular and multiseriate. The stigma produces only a limited amount of exudate or no visible exudate. In *Crotolaria* (Malti &

FIGURES 1–6. Scanning electron micrographs of stigma. —1. *Myroxylon peruiferum* (Sophoreae), voucher KAN 000-73.12286. —2. *Sophora japonica* (Sophoreae), voucher KAN 000-72.12999. —3. *Sophora macrocarpa* (Sophoreae), voucher KAN 398-73.05027. —4. *Wisteria sinensis* (Millettieae), voucher KAN 000-73.20755. —5. *Erythrina corallodendron* (Phaseoleae), voucher KAN 148-45.14801. —6. *Tephrosia bracetolata* (Millettieae), voucher KHSCN 15510. Stigma exudate scanty in Figures 1–3 and copious in Figures 4–6. Scale bars = 10 μm Figures 1, 2; = 100 μm Figures 3–6.

Shivanna, 1984) the secretion is confined to the base of papillae. In *Cicer* (Turano et al., 1983) it is confined to the interstices of a small cluster of papillae at the distal region of stigma; the remaining part of the stigma is free from secretion. In members of Aeschynomeneae and Mirbelieae (Lakshmi & Shivanna, 1985) and in Thermopsideae and Genisteae (this paper) there is no recognizable exudate on the stigma, at least at the level of the light and scanning electron microscope (Figs. 7–11; Table 4). The receptive surface may be obscured by nonreceptive hairs (Figs. 7, 8).

ORIGIN AND COMPOSITION OF EXUDATE

The details of the origin of the exudate have been studied in a few taxa that produce copious exudate. The stigma exudate is produced by the cells of the stigmatic surface as well as the subjacent 4–5 layers of the stigma (Ghosh & Shivanna, 1982; Heslop-Harrison & Heslop-Harrison, 1983). The stigma is composed of radially elongated rows of secretory cells with large intercellular spaces filled with secretion fluid. In *Vigna, Cajanus, Trifolium,* and *Glycine,* the cells of different rows remain interconnected at a number of points through plasmodesmatal connections, but in *Medicago* there are no such connections. In *Phaseolus* (Heslop-Harrison & Heslop-Harrison, 1984), three zones of glandular tissue are differentiated in the stigma head: an outer zone of a few palisadelike cell layers, an adjacent zone of compact tissue, and a zone of loosely arranged cells. The first zone produces a complex secretion containing proteins, polysaccharides, and lipids. The second zone



FIGURES 7–11. Scanning electron micrographs of stigma. —7. *Brachysema lanceolata* (Mirbelieae), voucher KAN 167-82.01356. —8. *Thermopsis divaricarpa* (Thermopsideae), voucher KAN 082-78.00727. —9. *Genista linifolia* (Genisteae), voucher KAN 458-67.45803. —10. *Cytisus villosus* (Genisteae), voucher KAN 156-82.01268. —11. *Laburnum anagyroides* (Genisteae), voucher KAN 393-71.03784. There is no recognizable stigma exudate. Scale bars = 100 μm.

secretes a viscous, nonlipidic component. The third zone also secretes nonlipidic but more fluid components.

The secretion fills the intercellular spaces of the stigma and eventually accumulates below the cuticle. The cuticle becomes detached from the surface cells by the accumulating exudate (Heslop-Harrison & Heslop-Harrison, 1983). This inflated cuticle is referred to as a "stigmatic membrane" in recent literature on plant breeding (see Free, 1970). In *Medicago*, however, the cuticle remains attached to the stigmatic cells at the outer tangential walls but stretches across intercellular spaces and confines the secretion. In many taxa the cuticle is disrupted before anthesis by the pressure exerted by the accumulating exudate, e.g., *Erythrina*, *Dalbergia*, and *Lablab* (Shivanna, unpublished), *Cajanus* (Ghosh & Shivanna, 1982), *Glycine* (Tilton et al., 1984), several lines of *Vicia faba* (Lord & Heslop-Harrison, 1984), species of *Medicago* (Kreitner & Sorensen, 1985), *Phaseolus* (Lord & Kohorn, 1986), and probably many other related taxa that do not require insect visits for effective pollination. In many others, the stigma cuticle remains intact until mechanical rupture during insect visitation (tripping), e.g., *Trifolium* (see Free, 1970), some lines of *Vicia faba* (Lord & Heslop-Harrison, 1984), species of *Medicago* (Kreitner & Sorensen, 1984), and *Lotus* (Bubar, 1958; Owens, 1985). The period of rupture of the cuticle has an important bearing on the breeding system of the species, and this aspect is discussed later.

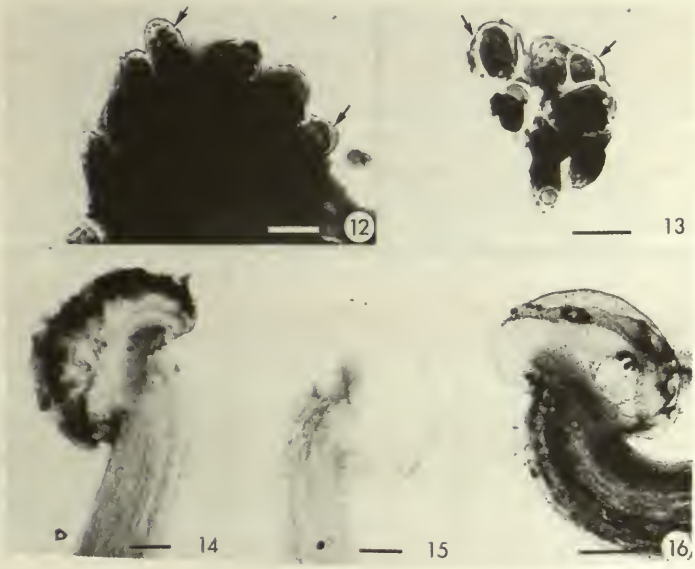
The details of the release of stigma secretion have not been studied in taxa producing limited amounts of secretion. In such species, the cuticle of the papilla does not become detached from the entire surface. As the cuticle seems to be very thin, particularly in the interstices of the papillae, the limited amount of secretion is presumably released through disruption of the cuticle between the papillae.

Some ultrastructural details of the cells of the stigma are available for *Trifolium* (Heslop-Harrison & Heslop-Harrison, 1983), *Medicago* (Kreitner & Sorensen, 1984, 1985), and *Glycine* (Tilton et al., 1984); all these produce copious exudate. During early development, the cells contain abundant ribosomes, smooth endoplasmic reticulum, numerous mitochondria and a well-developed golgi system—features characteristic of secretory cells. Stigmatic cells are heavily loaded with lipid droplets. However, the details of the extracellular secretion, particularly of the lipids, have not been studied. As the stigma matures, the secretory cells become moribund. The cuticle is two-layered; the inner layer is electron-opaque and the outer one homogeneous and less electron-opaque.

The stigma exudate is lipoidal (Bubar, 1958; Lord & Webster, 1979; Lord & Heslop-Harrison, 1984; Heslop-Harrison & Heslop-Harrison, 1984; Tilton et al., 1984; Kreitner & Sorensen, 1984, 1985; Lord & Kohorn, 1986) and viscous. It is held on the stigma surface by its viscosity as well as by a whorl of nonreceptive hairs present on the periphery of the stigma of many taxa (Webster et al., 1977). Apart from lipids, the exudate contains proteins, including nonspecific esterases and acid phosphatases (Ghosh & Shivanna, 1982; Heslop-Harrison & Heslop-Harrison, 1982a). In *Phaseolus*, the presence of amino acids, reducing acids, sugars, and phenols is also reported (Lord & Webster, 1979). In *Trifolium*, the principal sugars in the stigma secretion are sucrose and glucose, with traces of arabinose and galactose (Heslop-Harrison & Heslop-Harrison, 1982a). Microgradient gel electrophoretic analysis of the stigma secretion of *Trifolium* showed the presence of two major glycoproteins which are absent in the secretion of style. Differences between the secretions of the stigma and style have also been observed in the profiles of nonspecific esterases and acid phosphatases (Heslop-Harrison & Heslop-Harrison, 1982b).

TABLE 2. Reports of stigma-surface esterases in papilionoid legumes.

Tribe	Genus	References
Dalbergieae	*Dalbergia*	Shivanna (unpublished)
Millettieae	*Pongamia*	Shivanna (unpublished)
Indigofereae	*Cyamopsis*	Shivanna (unpublished)
Desmodieae	*Lespedeza*	Heslop-Harrison & Shivanna, 1977
Phaseoleae	*Cajanus*	Ghosh & Shivanna, 1982
	Erythrina	Shivanna (unpublished)
	Lablab	Shivanna (unpublished)
	Phaseolus	Heslop-Harrison & Heslop-Harrison, 1984; Lord & Kohorn, 1986
	Vigna	Ghosh & Shivanna, 1982
Psoraleeae	*Psoralea*	Heslop-Harrison & Shivanna, 1977
Aeschynomeneae	*Arachis*	Lakshmi & Shivanna, 1985
Galegeae	*Astragalus*	Heslop-Harrison & Shivanna, 1977
	Galega	Heslop-Harrison & Shivanna, 1977
Hedysareae	*Onobrychis*	Heslop-Harrison & Shivanna, 1977
	Hedysarum	Heslop-Harrison & Shivanna, 1977
Loteae	*Lotus*	Heslop-Harrison & Shivanna, 1977
Vicieae	*Vicia*	Heslop-Harrison & Shivanna, 1977
	Lathyrus	Lakshmi, 1984
Cicereae	*Cicer*	Malti & Shivanna, 1983; Turano et al., 1983
Trifolieae	*Medicago*	Heslop-Harrison & Shivanna, 1977
	Melilotus	Heslop-Harrison & Shivanna, 1977
	Ononis	Heslop-Harrison & Shivanna, 1977
Crotalarieae	*Crotalaria*	Malti & Shivanna, 1984
Genisteae	*Lupinus*	Heslop-Harrison & Shivanna, 1977



FIGURES 12–16. Whole mounts of stigma of *Arachis hypogaea* (dry stigma, Figs. 12, 13) and *Cajanus cajan* (wet stigma, Figs. 14–16), following localization of nonspecific esterases. —12. In addition to cytoplasmic esterases, pellicle of papillae also show esterase activity (arrows). —13. Papillae separated to show multicellular nature of papillae, and pellicle (arrows). —14, 15. Stigma from mature flowers incubated with substrate (Fig. 14) and without substrate (Fig. 15). Note esterase activity in the exudate (Fig. 14). —16. Stigma from flower, four days before anthesis (treated with substrate). Esterase activity is largely confined to membranous layer on the surface. Figures 12, 13 after Lakshmi & Shivanna, 1985; Figures 14–16 after Ghosh & Shivanna, 1982. Scale bars = 10 μm Figures 12, 13; = 100 μm Figures 14–16.

STIGMA-SURFACE ESTERASES

Since the demonstration of the presence of an esterase-positive pellicle overlying the cuticle in members of Cruciferae (Mattsson et al., 1974), the presence of surface esterases has been reported in a number of taxa of diverse families (see Shivanna & Johri, 1985). The pellicle is clearly seen in the dry type of stigma. In the wet type, particularly those producing copious exudate, a pellicle-cuticle layer generally gets disrupted during the release of exudate and, therefore, the pellicle is not clearly discernible in mature pistils. Stigmas of young pistils of many such species, however, do show an esterase-positive pellicle.

Table 2 gives a list of papilionoid legume genera in which esterases have been reported in the pellicle and/or the exudate. In species producing a limited amount of exudate or no apparent exudate, such as in *Crotalaria* (Malti & Shivanna, 1984), *Cicer* (Malti & Shivanna, 1983), and *Arachis* (Lakshmi & Shivanna, 1985), an extracuticular pellicle which responds to esterases can be clearly seen (Figs. 12, 13). The presence of pellicle has not been investigated in detail in species producing copious exudate, although esterases have been reported in the exudate of many taxa. In *Dolichos, Erythrina* (Shivanna, unpublished), *Cajanus,* (Figs. 14, 15) and *Vigna* (Ghosh & Shivanna, 1982), young stigmas (3–4 days before anthesis when the cuticle is still intact) show a thin surface membrane which responds to tests for esterases (Fig. 16). Even after the disruption of the stigma cuticle at the center of the stigma, an esterase-positive membranous pellicle is still visible in the peripheral region of stigma. However, in species of *Phaseolus,* esterase activity has been reported to be absent on the surface of intact cuticle (Heslop-Harrison & Heslop-Harrison, 1984).

III. The Style

The length of the style is highly variable and ranges from a few millimeters (*Cicer*) to 30–60 mm (*Arachis*). It may be straight, gently curved, or markedly reflexed. In most taxa the style is uniform in thickness throughout its length or it tapers gently from ovary to stigma. In taxa such as those classified in the subtribes Phaseolinae and Cajaninae of the tribe Phaseoleae, the style is thickened in the mid zone or towards the distal end to form a structure called the entasis (Table 3). The entasis may be variable both in size, shape, and position. In *Vigna adenanthus* and *V. venusta*, the entasis is a small, flattened thickening which is found about one-third the length of the style from the stigma. In *Dolichopsis paraguariensis*, the style is narrow for about one-third of its length, is thickened uniformly for the next third, and narrows once again for the final third. The junctions of the narrow and thick portions are abrupt. In *Dumasia villosa*, the style gradually thickens from the stigma, thickens abruptly at the point of flexure in the style, and is then very thin to the ovary. Little is reported regarding the anatomy of the entasis. In *Trifolium pratense*, Heslop-Harrison & Heslop-Harrison (1982b) indicate that where there is an entasis, the stylar canal is enlarged also. The style is glabrous in members of primitive as well as some advanced tribes. It is hairy in many of the advanced tribes. The hairs may be present throughout the length, or confined particularly to the upper part of the style (see Lackey, 1980).

Figures 17–55 present anatomical details of some representative taxa of different tribes. In all the taxa investigated so far, the style is basically similar. It is "hollow," with at least the upper part of the "canal" being derived lysigenously and with the lower part being continuous with the ovarian cavity. In none of the taxa, however, is the stylar canal continuous with the stigmatic surface. The glandular cells of the "solid" stigma converge at the base to form a strand of transmitting tissue with intercellular spaces (Fig. 17). This strand continues in the style to varying lengths, depending on species. A small stylar cavity appears in the center of the transmitting strand, just below the stigma, and gradually enlarges and extends throughout the length of the style. The transmitting tissue is confined to one or a few layers of cells, the canal cells, bordering the canal (Figs. 17–32).

In *Vigna unguiculata* (Ghosh & Shivanna, 1982), the solid core of transmitting tissue continues for 4–5 mm in the style. Transections of the style in this region are comparable to those of typical solid-styled taxa (Fig. 33). The core is made up of vertically elongated cells which appear circular in transection. The intercellular spaces are filled with secretion products that stain for proteins and carbohydrates. A small cavity appears further down the style in the center of the transmitting tissue as a result of degeneration and loosening of the central cells of transmitting tissue (Fig. 34). In transections, this region shows an irregular canal traversed by many loosely arranged cells of the transmitting tissue. The cells of the transmitting tissue at the periphery remain compact. The stylar canal gradually enlarges toward the base of the style, and the transmitting tissue is reduced to 2–3 layers of glandular cells (Figs. 35, 36). This structure is maintained throughout the remainder of the style. Transections in this region of style resemble those of typical hollow-styled taxa. There are no indications of cellular degeneration in this region. Thus, the canal in the upper region of style is secondarily derived during ontogeny by dissolution of the core of transmitting tissue, whereas the lower part of the style is typically hollow. This appears to be true in other papilionoid taxa also (Figs. 37–55; Lord & Kohorn, 1986).

Structural details of the upper part of the developing style in *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1982b) have given a clear picture of the lysigenous origin of the stylar canal in an advanced papilionoid. The style at a very young stage is typically solid, with a core of transmitting tissue. As the style matures, the cells of the transmitting tissue separate to form a canal, which receives secretions both from the core tissue and the inner wall cells. The secretion of proteins in early stages is associated with the presence of paramural bodies in adjacent cells. In later stages, proteins and carbohydrates accumulate in vesicles, probably derived from the golgi system,

TABLE 3. The occurrence of an entasis in genera of the Papilionoideae. An entasis may not be present in all species of the genus indicated, and its position on the style in relation to the stigma may vary considerably. Question mark indicates no data available on the presence or absence of an entasis.

Tribe Subtribe	Present	Absent
Swartzieae		—
Sophoreae		—
Dipterygeae		—
Dalbergieae		—
Abreae		—
Millettieae	*Kunstleria*	
	Tephrosia	— (other genera)
Robinieae		—
Indigoferae	*Rhynchotropis*	— (other genera)
Desmodieae	*Desmodium*	
	Droogmansia	
	Alysicarpus	— (other genera)
Phaseoleae		—
Erythriniae		—
Diocleinae		—
Glycininae	*Eminia*	
	Pseudeminia	
	Pseudovigna	
	Pueraria	
	Shuteria	
	Dumasia	?
Ophrestiinae		—
Kennediinae		—
Clitoriinae	*Centrosema*	
	Clitoriopsis	?
Phaseolinae	*Otoptera*	
	Phyostigma	
	Neurautanenia	
	Lablab	
	Dolichos	
	Vigna	
	Strophostyles	
	Macroptilium	
	Decorsea	
	Dolichopsis	
	Ramirezella	
	Phaseolus	
	Alistylus	
	Nesphostylis	?
Cajaninae	*Adenodolichos*	
	Cajanus	
	Atylosia	
	Dunbaria	
	Rhynchosia	
	Eriosema	
	Baukea	
	Endomallis	
	Flemingia	
	Chrysoscias	
	Paracalyx	?

TABLE 3. Continued.

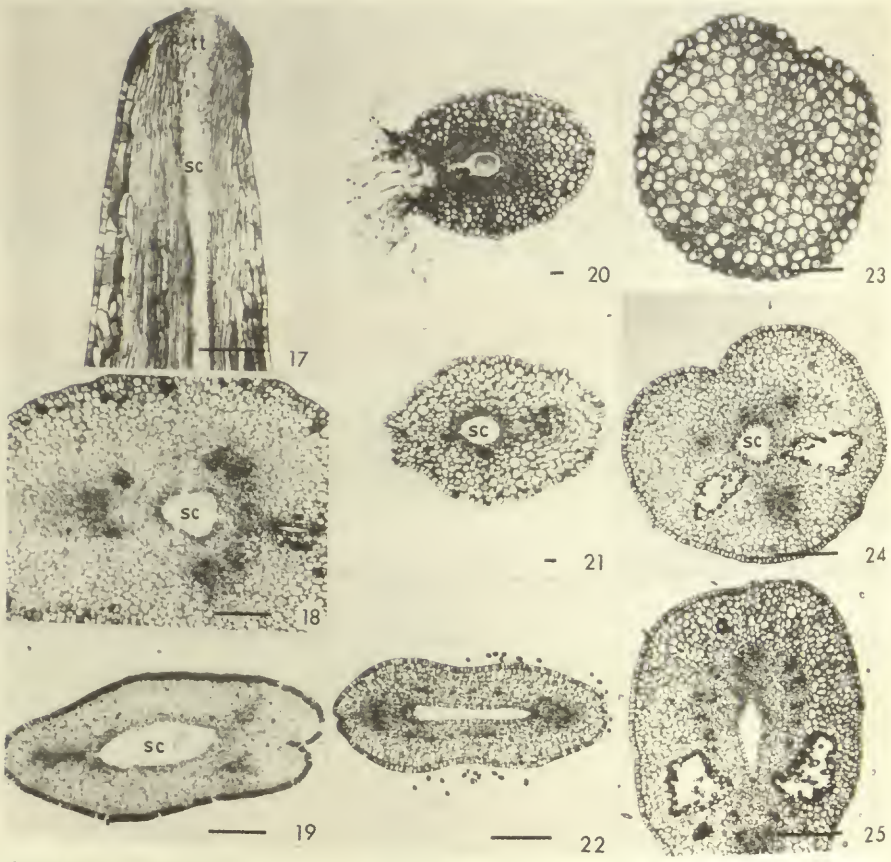
Tribe Subtribe	Present	Absent
Psoraleae	*Psoralea* *Hallia* *Otholobium* *Cullen* *Bituminaria* *Orbexilium*	
Amorpheae	?	?
Aeschynomeneae		—
Adesmieae	?	?
Galegeae		—
Carmichaelieae	?	?
Hedysareae		—
Loteae		—
Coronilleae	?	?
Vicieae		— (special structural changes—not entasis?)
Cicereae	?	?
Trifolieae	*Trifolium*	— (other genera)
Brogniartieae		—
Mirbelieae		—
Bossieae		—
Podalyrieae		—
Liparieae		—
Crotalarieae		—
Euchresteae		—
Thermopsideae		—
Genisteae		—

and form densely packed fibrillar nodules in the cytoplasm. They are dispersed into the canal following the dissolution of the cell membranes. Thus, two principal secretory processes, one involving paramural bodies (lomasomes) and the other the golgi system, seem to operate in the stylar secretion. Data is not available on the structure of style in the lower region.

In *Phaseolus acutifolius* (Lord & Kohorn, 1986), the upper part of style just below the stigma is also solid in the young bud. After further development, an ill-defined canal appears amidst the transmitting tissue due to cell degeneration. The rest of the style is typically hollow, lined with an epidermis covered by a very thin cuticle.

The canal cells show considerable variation in their structural details. In *Crotalaria juncea* (Ghosh & Shivanna, 1982), the wall of the canal cells appears absent in places, particularly on the side facing the canal. However, in other systems studied, the wall of the canal cells remains intact. In *Vigna unguiculata*, the canal cells are 2–3 layered (Fig. 35), and the inner tangential wall of the canal cells, particularly of the innermost layer, is thickened. In *Vigna radiata*, the canal cells are single-layered and do not show any thickenings. In places, however, the canal cells are absent; the canal in such regions is bordered by cells resembling cortical cells. In *Phaseolus acutifolius* (Lord & Kohorn, 1986), the transmitting tissue in the lower part of the style and ovary is confined to the ventral side of the canal. In *Crotalaria retusa*, the canal cells at the base of the style and upper 2–4 mm of the ovary protrude into the stylar cavity in the form of long papillae and loosely fill the cavity (Fig. 51) (Malti & Shivanna, 1984).

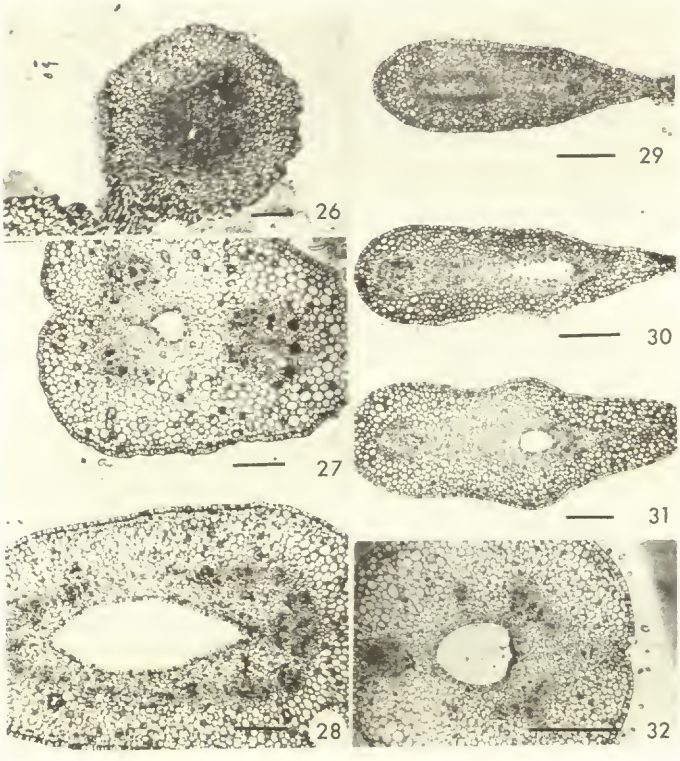
The canal cells in a majority of the taxa studied are glandular and densely cytoplasmic. They may



FIGURES 17–25. Sections of pistils. —17, 18. *Castanospermum australe* (Sophoreae). 17. Longisection of stigma and a portion of style. "Solid" stigma continues into the style (only in the upper region) as a core of transmitting tissue (tt); the rest of the style is hollow. sc = stylar canal. 18. Transection of style, middle region. Scale bars = 100 μm. (Voucher, Prakash & Cameron F 001.) —19. *Cladrastis kentukea* (Sophoreae). Transection of style. Scale bar = 100 μm. (Voucher, Prakash & Cameron F 007.) —20–22. *Dalbergia sissoo* (Dalbergieae). Transections of style at upper (Fig. 20), middle (Fig. 21), and lower (Fig. 22) regions. Stylar canal extends up to the region of stigma (Fig. 20). Scale bars = 10 μm Figures 20, 21; = 100 μm Figure 21. (Voucher, Shivanna 0013.) —23–25. *Pongamia glabra* (Millettieae). Transections of style below the stigma (Fig. 23), middle (Fig. 24), and lower (Fig. 25) regions. In *Pongamia* the style below the stigma is typically solid (Fig. 23). The middle and lower regions of the style are typically hollow in all the taxa. Scale bars = 100 μm. (Voucher, Shivanna 0014.)

be present all around the canal or restricted to a part of the canal. In *Arachis hypogaea*, however, although there is a well-defined layer of canal cells, they are nonglandular for almost the entire length of the style (Fig. 40; Lakshmi & Shivanna, 1985). They are vacuolate with peripheral cytoplasm and resemble cortical cells. The canal cells in the lowermost part of the style, however, are typically glandular, with dense cytoplasm and prominent nuclei comparable to those of other taxa.

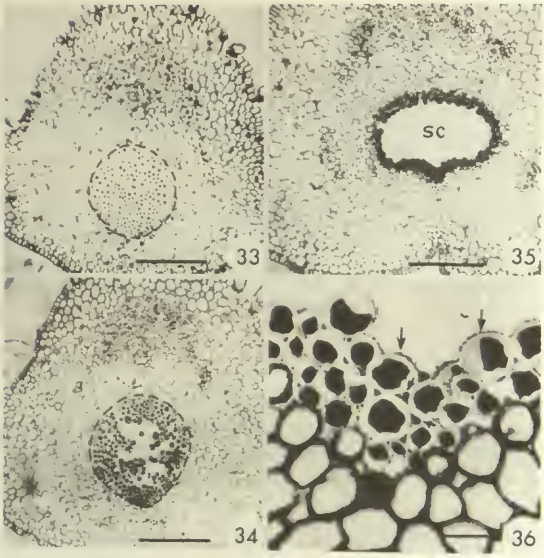
Ultrastructural details of the style are available for *Glycine max* (Tilton et al., 1984). The style has a solid core of transmitting tissue and the cells are free from one another along their axial walls.



FIGURES 26–32. Transections of style. —26–28. *Erythrina variegata* (Phaseoleae). Transection at upper (just below the stigma, Fig. 26), middle (Fig. 27), and lower (Fig. 28) regions. (Voucher, Shivanna 0018.) —29–32. *Lablab purpureus* (Phaseoleae). Transection at upper (Fig. 29), middle (Figs. 30, 31), and lower (Fig. 32) regions. The initiation of a canal is visible in sections below the stigma, and it gradually enlarges. In the middle and lower regions, it is bordered by an epidermal layer. In *Lablab* (Fig. 32) the glandular cells are restricted to only one side of the canal, in the basal region of the style and the upper part of the ovary. Scale bars = 100 μm.

Intercellular spaces are filled with the secretion product. The cell wall exhibits three layers—a diffuse outer layer integrating with the secretion product filling the intercellular space, a middle layer made up of a reticulum of loosely woven fibers, and an inner electron-dense layer. The cytoplasm of the cells is dense and rich in polysomes, mitochondria, plastids, and rough endoplasmic reticulum. Many of the plastids contain starch grains and small lipid droplets. It is not clear from the paper (Tilton et al., 1984) whether the style is solid only in the upper part (as in species of *Vigna*) or whether a solid transmitting tissue extends throughout the entire length of the style. If it is solid throughout, this is the only report of a typically solid style in the papilionoid legumes so far investigated.

The contents of the stylar canal have been analyzed in *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1982a). The canal is filled with a watery secretion throughout its length. Electrophoretic analysis of secretion from the region of the entasis has shown distinct differences in the banding pattern of proteins, esterases, and acid phosphatases when compared to those of the stigma secretions. In *Cicer* (Malti & Shivanna, 1983), a fluid secretion is recognizable only in the upper part of the style. In *Vigna unguiculata* (Ghosh & Shivanna, 1982), anatomical studies of fixed

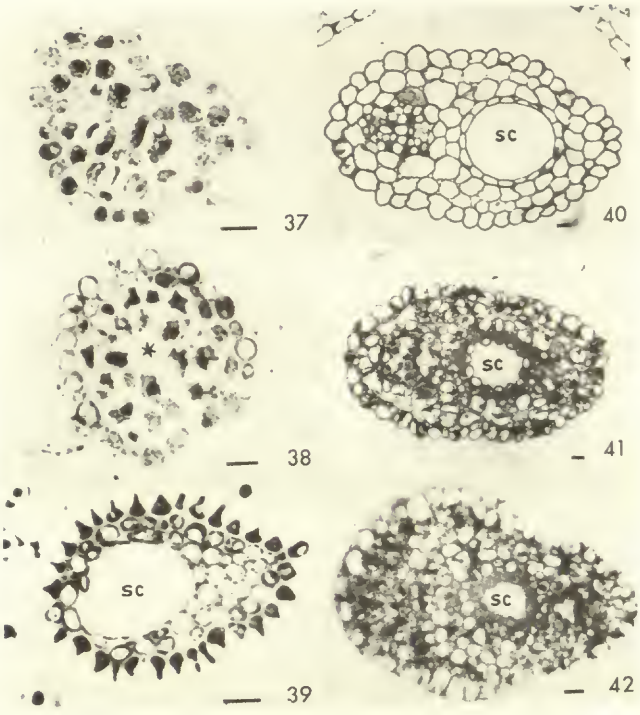


FIGURES 33–36. *Vigna unguiculata* (Phaseoleae) style transections. —33. Four to five mm below the stigma. A solid core (dashed circle) of transmitting tissue occupies the central area. —34. Six to eight mm below the stigma. There is an irregular stylar cavity at the center of the transmitting tissue (dashed circle). —35. At the base of the style. The style is typically hollow, with a large stylar canal (sc) bordered by two to three layers of canal cells. —36. Part of a section comparable to Figure 35, stained with Coomassie blue. Note extracellular proteins in the intercellular spaces (short arrows) of canal cells and as a lining facing the canal (long arrows). After Ghosh & Shivanna, 1982. Scale bars = 100 μm Figures 33–35; = 10 μm Figure 36.

material did not reveal any fluid secretions in the canal. A layer of proteinaceous secretion is present on the inner wall facing the stylar canal (Fig. 36). In *Crotolaria retusa* (Malti & Shivanna, 1984), the canal is free from fluid secretion. As in *Vigna*, a layer of extracellular proteins is present on the inner side of the canal cells facing the cavity (Fig. 50). In *Phaseolus acutifolius* (Lord & Kohorn 1986), the cells of the transmitting tissue are restricted to the ventral side, and the secretion originating from these and the underlying cells covers the surface of transmitting cells. In some taxa such as *Cajanus* (Ghosh & Shivanna, 1982) and *Arachis* (Lakshmi & Shivanna, 1985), neither a fluid secretion nor a distinct proteinaceous layer was observed following the conventional preparation of the fixed material at the light microscopic level. Ultrastructural studies of the canal cells have not been undertaken.

IV. Taxonomic and Evolutionary Relationships

The subfamily Papilionoideae comprises some 440 genera (Polhill, 1981). Of these, some information, more often incomplete, is available on the nature of the stigma and style of only 45 genera belonging to 18 tribes. Also, the number of genera investigated in each tribe is one or, at the most, only a few. Although these studies have brought out considerable similarities and variations in morphological and anatomical features of the stigma and style, the data is insufficient to indicate evolutionary trends. It is nevertheless feasible to examine the variations in the stigma and style in the light of relationships of different tribes already suggested on the basis of distribution, morphology, cytology, anatomy, and phytochemistry (Polhill, 1981; Polhill et al., 1981).

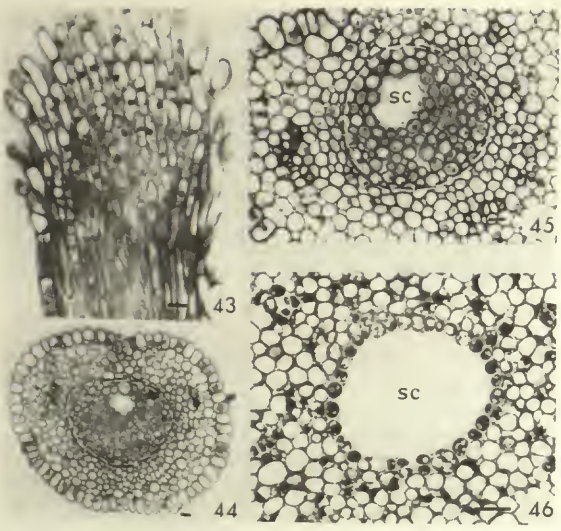


FIGURES 37–42. *Arachis hypogaea* (Aeschynomeneae) stigma and style transections. —37. Base of stigma, showing cells of transmitting tissue with large intercellular spaces formed by converging stigmatic papillae. —38. Just below the stigma. Asterisk indicates a small canal in the center of the transmitting tissue. —39. At a lower level than in Figure 38. The stylar canal (sc) has enlarged and is not lined with canal cells. —40. Middle portion. Stylar canal lined with a layer of canal cells resembling cortical cells. —41, 42. Lowermost part of style. Canal cells filled with dense cytoplasm and prominent nuclei. After Lakshmi & Shivanna, 1986. Scale bars = 10 μm.

Table 4 presents some salient features of the stigma and style in different tribes of the Papilionoideae. It is evident that many tribes show uniformity in most of the features. Differences within the tribes are confined to the papillate or nonpapillate nature of stigma and in the presence or absence of nonreceptive hairs at the base of the stigma and on the style.

In members of the Sophoreae, considered to be one of the most primitive tribes, the stigma is least differentiated. It is small and inconspicuous at the tip of a slender style. The stigma is wet, papillate or nonpapillate, and the amount of stigmatic secretion is limited. The stigma is free from basal nonreceptive hairs, and there is no indication of the presence of a stigmatic membrane (formed by lifting of the cuticle on the entire receptive surface). The style is slender and glabrous. There are no adaptations to prevent self-pollen coming in contact with the stigma. Self-incompatibility, when present, seems to be the only possible device to prevent inbreeding.

A major evolutionary trend seems to be toward the differentiation of a well-developed stigma. The stigma may be smooth or papillate; the papillae (when present) are generally short. The stigma produces copious exudate, lifting the cuticle off the entire surface in the form of a membrane. The membrane may get disrupted during development or remain intact until tripping. When it remains intact, the membrane acts as a barrier for self-pollen. This type of stigma is present in a number of tribes such as Dalbergieae, Millettieae, and Indigofereae, including many of the advanced tribes



FIGURES 43–46. *Cicer arietinum* (Cicereae). —43. Longitudinal section of stigma. —44–46. Transections of the style at successively lower levels from the stigma, showing stylar canal (sc), which enlarges toward the base. Dashed circles demarcate the boundary of central transmitting tissue. The stylar canal in Figure 46 is lined with a layer of glandular cells. After Malti & Shivanna, 1983. Scale bars = 10 μm.

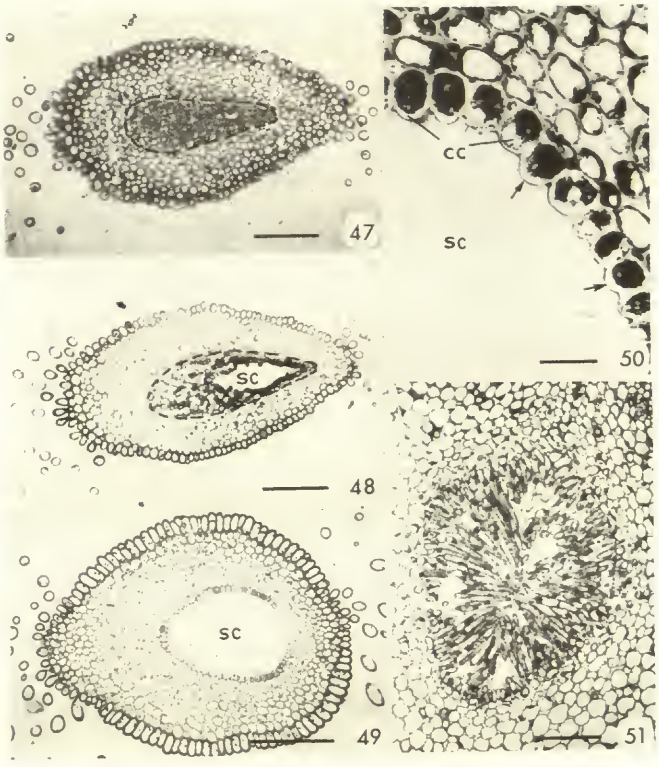
such as Phaseoleae, Trifolieae, and Loteae. In many of these taxa the stigma bears a whorl of nonreceptive hairs, which may also act as a barrier to self-pollen. Further elaboration is seen in the development of nonreceptive hairs on the style. Although there are variations between taxa in the shape of the stigma, and the presence of nonreceptive hairs at the base of stigma and on the style, these characters seem to have evolved independently in different tribes and do not indicate the relationships of the tribes. The bearded style, for example, seems to have evolved at least three times in the tribe Phaseoleae itself (Lackey, 1981). However, these characters are extremely useful in taxonomic delimitation of related genera (e.g., see Gunn & Kluve, 1976; Small & Brookes, 1983).

Another evolutionary trend in the stigma seems to be toward reduction in the amount of secretion produced. This feature is prevalent in members of many advanced tribes such as Cicereae, Crotalarieae, and Genisteae. The limited amount of exudate produced remains confined to the interstices of papillae and does not result in the lifting of the cuticle in the form of a membrane. In members of the Aeschynomeneae, there is no visible exudate, and the stigma appears to be dry. This feature is a derived condition, as many of them still retain other advanced features, particularly the presence of hairs at the base of the stigma and on the style.

The anatomy of the style appears to be rather uniform throughout the Papilionoideae, being basically intermediate between solid and hollow style types. Although in the mature pistil the stylar canal extends for almost the entire length of the style, the upper part is derived lysigenously from the solid part of the style during ontogeny. There are variations in the extent of a lysigenously formed canal and in the nature and distribution of glandular cells in the basal region of the stylar canal, but the available data does not indicate any clear pattern in these variations.

V. Stigma Receptivity and Pollination

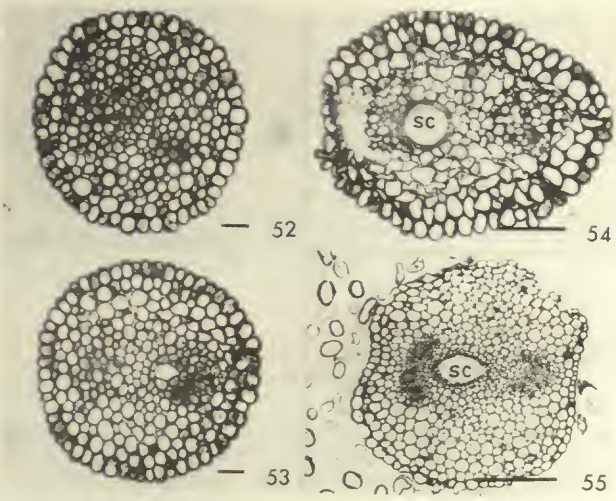
The period when the stigma becomes receptive varies from species to species. In *Cicer* (Malti & Shivanna, 1983), *Crotalaria* (Malti & Shivanna, 1984), and *Arachis* (Lakshmi, 1984), which



FIGURES 47–51. *Crotalaria retusa* (Crotalarieae) style transections. —47. At the base of stigma. A strand of transmitting tissue (dashed circle) fills the central part, and the stylar cavity has not yet developed. —48. Just below stigma. On one side of stylar canal (sc) there is a considerable amount of transmitting tissue (dashed circle). —49. Middle part of style. The stylar canal is larger than in Figure 48, and the tranmitting tissue has become reduced to a single layer bordering the canal. —50. Part of a section (comparable to Fig. 49) stained with Coomassie blue. The canal cells (cc) are richly proteinaceous, and a thin proteinaceous layer is also seen on the wall facing the canal (arrows). —51. Base of style, tip of ovary. The canal is loosely filled with long unicellular papillae formed by the elongation of canal cells. After Malti & Shivanna, 1984. Scale bars = 100 μm Figures 47–48, 51; = 10 μm Figure 50.

produce little or no exudate, the stigma becomes receptive 1–3 days before anthesis. In these taxa the pollen grains seem to be capable of hydration and germination even in the absence of exudate. The stigmatic cuticle therefore does not appear to act as a barrier for hydration. Presumably, in these species the cuticle has discontinuities, as has been shown in many dry types of stigmas, permitting the passage of water from the stigmatic papillae to pollen.

In taxa that produce copious exudate, stigma receptivity depends on the state of the stigmatic cuticle. In those in which the cuticle gets disrupted before anthesis, releasing the exudate onto the surface, the stigma becomes receptive soon after disruption of the cuticle, generally 1–2 days before anthesis. In species in which the cuticle remains intact until flower opening, the stigma becomes receptive only after tripping. In all the taxa which produce copious exudate, availability of exudate seems to be essential for pollen hydration and germination (Armstrong & White, 1935; Brink & Cooper, 1936; Toynbee-Clarke, 1971; Paul et al., 1978; Lord & Heslop-Harrison, 1984). In *Trifolium* (Heslop-Harrison & Heslop-Harrison, 1983), *Vicia* (Lord & Heslop-Harrison, 1984),



FIGURES 52–55. *Lupinus* sp. (Genisteae) transections just below the stigma (Fig. 52) and of the style (Figs. 53–55), showing the initiation and enlargement of the stylar canal (sc), which is bordered by a layer of canal cells in the middle (Fig. 54) and lower (Fig. 55) regions of the style. Scale bars = 10 μm Figures 52, 53; = 100 μm Figures 54, 55.

TABLE 4. Analysis of the types of stigma and style in different tribes of papilionoid legumes. WP = wet, papillate; WN = wet, nonpapillate; DP = dry, papillate; C = copious; S = sparse; H = hollow; + = present; − = absent; 0 = no data.

Tribe (Number of genera studied)	Stigma				Style	
	Morphology	Exudate	Membrane	Basal hairs	Hairs	Anatomy
Swartzieae (1)	0	0	0	0	0	H
Sophoreae (4)	WP/WN	S	0	−	−	H
Dalbergieae (1)	WN	C	+	−	−	H
Millettieae (3)	WN	C	+	−/+	−	H
Indigofereae (2)	WP/WN	C	+	+/−	−	H
Phaseoleae (7)	WP/WN	C	+	+/−	+/−	H
Aeschynomeneae (3)	DP	−	−	−	+/−	H
Galegeae (3)	WP/WN	S	0	−	+/−	H
Loteae (1)	WP	C	+	−	−	0
Coronilleae (2)	WP	S	−	+	0	0
Vicieae (2)	WN/WP	C	+	−	+	H
Cicereae (1)	WP	S	−	−	+	H
Trifolieae (2)	WN	C	+	+/−	−	0
Mirbelieae (3)	DP	−	−	−	−	H
Bossiaeeae (1)	0	0	−	−	−	H
Crotalarieae (1)	WP	S	−	+	+	H
Thermopsideae (1)	DP	−	−	−	0	0
Genisteae (5)	DP/WP	−/S	−	+/−	−	H

Phaseolus (Heslop-Harrison & Heslop-Harrison, 1984), and *Lotus* (Owens, 1985), pollen grains deposited on an intact stigma cuticle (before the release of exudate) fail to hydrate and germinate. However, when the cuticle is perforated mechanically, releasing the exudate, pollen grains readily

hydrate and germinate (Heslop-Harrison & Heslop-Harrison, 1984). In these systems, the cuticle probably acts as a barrier for the passage of water.

In most papilionoid legumes, the anthers and stigma are closely juxtaposed in the flower, and thus the pollen grains readily come in contact with the stigma soon after the anthers dehisce. Cleistogamy is reported in many papilionoid legumes (see Arroyo, 1981; Lord, 1981). The details of the pollen–pistil interaction in cleistogamous flowers have been investigated in many species of *Lespedeza* (Hanson, 1953; Hanson & Cope, 1955). Cleistogamous flowers are generally small, and the style is strongly hooked, bringing the stigma in contact with one or more anthers. Pollen grains germinate within closed anther sacs, and the pollen tubes penetrate the anther wall and enter the stigma. In chasmogamous flowers, the extent of self-pollination is highly variable, often in different cultivars of the same species. Some taxa are largely autogamous, but in some others insect visits (tripping) are essential for effective pollination. In still others, although selfing does occur to some extent, tripping considerably increases fruit- and seed-set. The details of different species belonging to these categories are well-documented (Whealer & Hill, 1957; Free, 1970; McGregor, 1976). Even in species that are dependent on tripping, the extent of selfing varies; species may be self-fertile, moderately self-fertile, or fully self-incompatible. In *Lupinus palestinus* and *L. pilosus* (Pazy, 1984), for example, although pollination is dependent on tripping, self-fertility is high and almost the entire progeny is the result of selfing. It is suggested that retention of dependence on insects for pollination provides an avenue for occasional outbreeding.

Only limited studies have been carried out to correlate the structural features of the flower, particularly the stigma, with the pollination biology of the species. These studies are largely confined to the cultivars that produce a copious stigmatic exudate. There are three basic mechanisms that prevent self-pollination and fertilization: (i) the architecture of the flower, particularly the pistil, which prevents the pollen coming in contact with the stigma; (ii) the state of the stigmatic cuticle; and (iii) self-incompatibility. More than one of these features often occur in the same species, and it is their combination which determines the extent of cross-pollination. In some cultivars of *Vicia faba,* Kambal et al. (1976) reported that variation in papillar length and disposition, in conjunction with other structural features of the flower such as stylar length, number and length of stylar brush hairs, and morphology of the inner surface of the keel petals, could be effective in controlling the access of pollen to the stigma surface. In many species the whorl of long, nonreceptive hairs present at the base of the stigma may be effective in preventing the pollen coming in contact with the stigma.

It has been known since the time of Jost (1907) that in many papilionoid legumes the stigma is covered by a stigmatic membrane (cuticle), and that the rupture of this "membrane" is essential for pollen germination (see Free, 1970). In recent years the details of the stigmatic cuticle have been investigated in a few systems. Some species or lines, termed autofertile, are largely inbreeders; effective pollination occurs without tripping or mechanical disturbances. Other lines, termed autosterile, require tripping for effective pollination. In autosterile lines, cross-fertilization is reported to be as high as 40% (Toynbee-Clarke, 1971). Recent studies by Lord & Heslop-Harrison (1984) on *Vicia faba* have shown that the organization and mechanical strength of the stigma cuticle is a major factor in governing autofertility in different lines. An autofertile line has low papillae with a relatively thin intervening cuticle (130 nm); the cuticle gets disrupted (without tripping) before flower opening and releases the exudate onto the stigma surface. A strongly autosterile line, on the other hand, has longer papillae and a thicker cuticle (400 nm). The cuticle remains intact even when the flower opens, and thus the exudate remains below the cuticle. Such stigmas do not support pollen germination. Tripping by pollinating insects, however, results in the rupture of the cuticle and the release of exudate. A line with partial autofertility was intermediate in the length of papillae and the thickness of the cuticle (220 nm). Apart from the thickness of cuticle, the release of exudate is also, to some extent, affected by environmental conditions, particularly the water status of the plant. With sufficient moisture, increased stigma secretion may induce rupture of the cuticle even in the autosterile plants by exerting increased pressure on the cuticle. Water stress, on the other

hand, may reduce the amount of secretion, which is insufficient to disrupt the cuticle even in autofertile plants (see Lord & Heslop-Harrison, 1984). A similar situation occurs in *Medicago*. In autofertile *Medicago scutellata* the cuticle is thin (ca. 75 nm) and is invariably disrupted during the late stages of flower development (Kreitner & Sorensen, 1985). In autosterile *M. sativa*, however, the cuticle is thick (ca. 120 nm) and remains intact until tripping, acting as a barrier between self-pollen and stigmatic fluid.

A comparable stigmatic membrane is absent in genera such as *Arachis*, *Cicer*, and *Crotalaria*, which produce very little or no visible exudate. In *Arachis* and *Cicer*, the stigma is not covered by a whorl of nonreceptive hairs at the base, and thus there is no morphological barrier preventing the self-pollen from reaching the stigma. In such systems the only mechanism that can prevent selfing is self-incompatibility. Both *Arachis* and *Cicer* species are self-compatible and largely autogamous.

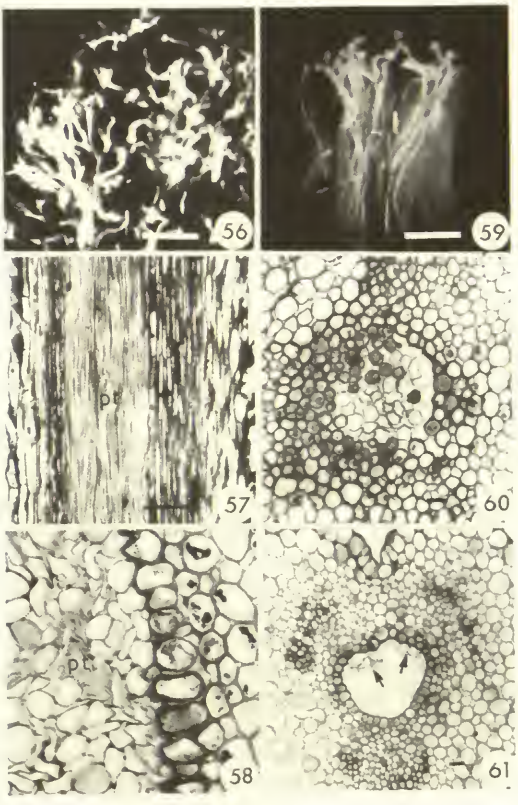
In *Crotalaria* there is a dense whorl of nonreceptive hairs at the base of stigma, which to some extent prevents pollen grains coming in contact with the stigma. As the stigma exudate is confined to the basal region of papillae, pollen grains, even when they land on the stigma, do not make effective contact with the exudate. *Crotolaria*, as well as many other species that bear a whorl of nonreceptive papillae at the base of the stigma, requires tripping. The role of tripping in bringing about effective pollination in such systems is not clear. Presumably, tripping helps bring pollen grains in contact with the stigma surface as well as in pushing the pollen between the papillae to bring them in contact with the exudate. Tripping may also cause mechanical damage to the stigma papillae, which may promote pollen germination and/or pollen tube entry into the stigma.

In *Vicia faba* there is a correlation between the ability to self and heterozygosity (Drayner, 1956, 1959; Holden & Bond, 1960). Heterozygous plants can self, but inbred plants require tripping, and their progeny therefore contain a higher proportion of outbred individuals. This acts as a feedback system regulating inbreeding. In winter beans (*Vicia faba* L. var. *exina*) the stigma surface of highly inbred lines gave better seed-set following emasculation and hand pollination when compared to tripping (Toynbee-Clarke, 1974). It is suggested that inbreeding makes the stigmatic papillae more resistant to breakage and penetration of pollen tubes (presumably by thickening of the cuticle); emasculation and hand pollination treatments inevitably produce some degree of scarification, resulting in more effective pollination and better seed-set.

VI. Pollen Tube Growth

The entry of the pollen tube into the stigma has been studied in only a few systems. In species in which the stigmatic membrane gets disrupted before pollen germination, pollen tubes enter the stigma head through the torn gaps of the cuticle and not by enzymatic digestion of the cuticle. The details of pollen tube entry have not been studied in systems in which the stigmatic cuticle is absent or ineffective. After entering the stigma, the pollen tubes grow through intercellular spaces in the transmitting tract and eventually enter the stylar canal (Figs. 56–59). In *Crotalaria* (Malti & Shivanna, 1984), tubes often fill the entire stylar canal (Figs. 57, 58). In such pistils the bundle of pollen tubes can be easily dissected and separated from the rest of the stylar tissue. Following pollen tube growth, the canal cells show signs of vacuolation and degeneration (Fig. 58). In some species of *Phaseolus* the pollen tubes are reported to grow close to the vascular tissue in the style (Hawkins & Evans, 1973). Recent studies by Lord & Kohorn (1986) on *P. acutifolius* have shown that pollen tubes in the style are restricted to the transmitting tract (present only on the ventral side of the stylar canal). The pollen tubes are never seen free in the canal.

In recent years, the role of the pistil in screening even compatible pollen tubes has been emphasized (Mulcahy, 1979). The pistil is believed to intensify competition among pollen tubes, favoring only the most vigorous ones for effecting fertilization. A few studies on pollen tube growth in papilionoid legumes provide evidence for such a function of the pistil. In *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1982a), pollen tubes freely enter the region of the entasis.



FIGURES 56–61. Pollen tube growth. —56–58. *Crotalaria retusa* (after Malti & Shivanna, 1984). —59–61. *Cicer arietinum* (after Malti & Shivanna, 1983). Whole mounts of pollinated stigma stained with decolorized aniline blue, showing entry of a large number of pollen tubes into the stigma (Figs. 57, 60). 57. Longisection of a part of pollinated style. Pollen tubes (pt) fill the entire stylar canal. 58. Central part of transection of pollinated style, showing pollen tubes (pt) in the stylar canal. The canal cells are vacuolate and show signs of degeneration. 60, 61. Transections of middle (Fig. 60) and lower (Fig. 61) region of pollinated style, with pollen tubes in the canal. Pollen tubes are fewer (arrows) in the lower region. Scale bars = 100 μm Figures 56, 57, 59; 10 μm Figures 58, 60, 61.

However, only a few tubes extend beyond this zone in the style, and the successful ones continue their growth into the ovary. In *Crotalaria retusa* (Malti & Shivanna, 1984), although a large number of pollen tubes enter the papillate region in the upper part of the ovary, only a limited number of pollen tubes were found to grow beyond this region into the ovulate part of the ovary. Thus, pollen tubes are subjected to screening in these specialized regions of the style.

In *Cicer* (Malti & Shivanna, 1983) and *Glycine max* (Tilton et al., 1984), most of the pollen grains deposited on the stigma germinate and produce tubes that grow down into the stigma and upper part of the style. The majority of pollen tubes (up to 90%), however, cease growth before they reach the base of style or the upper part of the ovary (Figs. 60, 61); only a few reach the locule and effect fertilization. This has been attributed to pollen tube competition in the style, resulting in the selection of more vigorous pollen tubes reaching the ovules. In *Phaseolus acutifolius* also, a large number of pollen tubes penetrate the stigma, but very few tubes are seen in the stylar canal. As the transmitting tissue in this taxon is restricted to the ventral side of stylar canal, it can accommodate

only a limited number of tubes, thus providing a possible site for selection of male gametes (Lord & Kohorn, 1986).

In *Crotolaria retusa* (Malti & Shivanna, 1985), incorporation of leachates from bissected pistils in the in vitro pollen germination medium significantly increases the number of pollen tubes in the lower frequency range (for tube length) and decreases those in the medium frequency range. A small proportion of tubes grow much beyond the maximum length attained in the control medium. Thus, the pistil leachates stimulate the growth of a limited number of pollen tubes, giving them an advantage over others in effecting fertilization.

VII. Incompatibility

In papilionoid legumes, self-incompatibility (SI) is reported in 71 out of 321 species tested (Arroyo, 1981). It is present only in a few cultivated species, in genera such as *Lotus*, *Trifolium*, and *Medicago*. Considerable studies on SI have been carried out (particularly on the genetic control), and these have been comprehensively reviewed by Townsend (1971). Self-incompatibility is gametophytically controlled and is governed by multiple alleles at one locus (Williams & Silow, 1933; Williams, 1947a, b; see also Townsend, 1971). In general, self-incompatibility does not affect pollen germination, but pollen tube growth is inhibited in the style. In species of *Trifolium* (Atwood, 1941) and *Medicago* (Sangduen et al., 1983), however, self-incompatibility is also reported to inhibit pollen germination. In *Trifolium pratense*, incompatible pollen tubes are inhibited just beyond the region of the entasis (see Heslop-Harrison & Heslop-Harrison, 1982a). Incompatible tubes often show many abnormalities such as coiling, swelling, and branching (Sangduen et al., 1983). Environmental factors, particularly temperature, affect self-incompatibility (see Townsend, 1971). In *Trifolium*, different clones showed variability in response to high temperature. In temperature-sensitive clones, increase in temperature decreased the self-incompatibility reaction and thus increased seed-set following selfing. Information is not available on the nature of components involved in this self-incompatibility. In *Trifolium pratense* a glycoprotein component with a molecular weight of 24,000 daltons is present in the stylar fluid in which self-pollen tubes are inhibited (Heslop-Harrison & Heslop-Harrison, 1982a). This glycoprotein was absent in the stigma elute. However, the role of the glycoprotein in the self-incompatibility response remains to be studied.

In many systems self-incompatibility is not fully effective in preventing fertilization by self-pollen, thus ensuring some seed-set in the absence of cross-pollen. For example, in *Lotus corniculatus* (Bubar, 1958), which requires tripping for the breakdown of the stigmatic cuticle, self-pollen tubes enter the ovary less readily than cross-pollen tubes. When cross-pollen becomes available during the first insect visit, both self- and cross-pollen grow simultaneously. Eventually, cross-pollen succeeds in fertilization. If cross-pollen is not available, self-pollen would still induce seed-set. Another interesting feature concerning self-incompatibility, reported in different species of *Lotus* (Bubar, 1958; Owens, 1985), is the variability in the development stages of different ovules in the same ovary. Whereas in self-fertile species all ovules within each ovary develop uniformly, in SI species they show considerable variation in the rate of development. All ovules in SC species may be fertilized, while less than one-half of those in SI species are generally fertilized. It is suggested that the loss of uniformity in ovule development in SI species is to compensate for variability in the time of insect pollination (Bubar, 1958).

In self-incompatible clones of *Medicago sativa* (Sangduen et al., 1983), although fewer self-pollen grains germinated, and self-pollen tubes grew slowly, over 20% of the ovaries contained at least one fertilized ovule in self-pollinated pistils. However, they failed to develop into seeds. In this species, therefore, both pre- and postfertilization barriers seem to prevent selfing.

Although there have been many attempts to obtain interspecific hybrids in different papilionoid legumes (see Singh, 1981; Newell & Hymowitz, 1983; Sastri, 1984), studies on pollen germination

and pollen tube growth following interspecific crosses are limited. In *Medicago* the time between pollination and fertilization for interspecific crosses is about double when compared to compatible pollinations (Sangduen et al., 1983). In *Arachis,* compatible pollen tubes had small and evenly distributed callose plugs, while incompatible tubes showed large and irregularly distributed plugs (Sastri & Moss, 1982). *Phaseolus vulgaris* is self-compatible, while *P. coccineus* is self-incompatible. Self pollen tubes in the latter are inhibited in the stigma head or in the lysigenous stylar canal (Heslop-Harrison & Heslop-Harrison, 1984). Interspecific crosses of these species exhibit unilateral incompatibility. The pollen of *P. coccineus* can traverse the stigma and style of *P. vulgaris.* The pollen of *P. vulgaris,* however, is inhibited in the tissue of the stigma head or in the lysigenous canal of *P. coccineus* in a similar manner to self-pollen.

VIII. Prospects

As pointed out earlier in this paper, investigations on pollen, stigma, and style interactions in papilionoid legumes have so far been fragmentary and cover only a limited number of taxa, largely the cultivated species. Experimental studies are practically absent. Even ultrastructural studies have only just been initiated. There is no information on Dipteryxeae, Abreae, Robinieae, Desmodieae, Psoraleeae, Amorpheae, Adesmieae, Carmichaelieae, Hedysareae, Brongniartieae, Podalyrieae, Liparieae, and Euchresteae. The data particularly on pollen–pistil interaction is very meager. In addition, there are no comprehensive studies on the details of secretion and the nature of secretory cells in the style. For a better understanding of the structure of the pollen and pistil in relation to their function, in-depth studies covering all the tribes are necessary.

Apart from studies aimed at understanding the biology of the pollen–pistil interaction, comprehensive investigations on the structural aspects of the pistil of a large number of taxa within a tribe (as has been done on *Medicago* and related genera—Small & Brookes, 1983) would provide a wealth of information on taxonomic relationships of the species and genera.

As in other crop plants, so in papilionoid legumes, adverse environmental conditions significantly affect seed-set (see van Stevenick, 1957; Bond & Poulsen, 1983; Gates et al., 1983). However, there are very few studies on their effect on the pollen–pistil interaction. In *Cicer,* differences in pollen germination and pollen tube growth in different cultivars have been correlated with differences in the ability of the cultivars to set seed under low temperature (Savithri et al., 1980). Such studies are important for agronomists and plant breeders. There are positive correlations (in some systems) between the performance of pollen and that of the sporophyte, particularly under unfavorable conditions such as the presence of pathotoxins, adverse temperature, and salinity. In a few systems it has been feasible to use pollen as a system to identify sporophytes resistant to adverse environmental conditions (Mulcahy, 1984). The pollen from a single plant can be used to test against a range of environmental conditions, which greatly reduces the time and cost of screening genotypes for desirable characters. It is also feasible to increase the frequency of desirable genes in the progeny (Zamir et al., 1981; Sacher et al., 1983). On these lines, studies on legumes are yet to be initiated and should be encouraged, given the economic importance of legumes.

Although flower abscission is one of the major problems in the improvement of food legumes (see Sinha, 1977), serious studies have not been undertaken to determine the role of pollination and pollen–pistil interaction, if any, in flower abscission (see Stephenson, 1981, for an overall review of this phenomenon in angiosperms). Preliminary studies carried out on these aspects on *Vigna* (Ojehomon, 1972, 1975) and *Vicia* (Marcellos & Perryman, 1986) indicate deviations in both prefertilization and postfertilization processes in the abscissing flowers or pods. Such investigations need to be extended to characterize the pollen–pistil interaction and fertilization in relation to pod production.

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References

Armstrong, J. M. & W. J. White. 1935. Factors influencing seed set in alfalfa. J. Agric. Sci. (Cambridge) 25: 161–179.

Arroyo, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Atwood, S. S. 1941. Cytological basis for incompatibility in *Trifolium repens*. Amer. J. Bot. 28: 551–557.

Bond, D. A. & M. H. Poulsen. 1983. Pollination. Pp. 77–101 *in* P. D. Hebblethwaite (editor), The Faba Bean (*Vicia faba* L.). Butterworths, London.

Brink, R. A. & D. C. Cooper. 1936. The mechanisms of pollination in alfalfa (*Medicago sativa*). Amer. J. Bot. 23: 678–683.

Bubar, J. S. 1958. An association between variability in ovule development within ovaries and self-incompatibility in *Lotus* (Leguminosae). Canad. J. Bot. 36: 65–72.

Drayner, J. M. 1956. Regulation of outbreeding in field beans (*Vicia faba* L.). Nature 177: 489–490.

———. 1959. Self fertility in field beans (*Vicia faba* Linn.). J. Agric. Sci. 41: 371–378.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, London & New York.

Gates, P., M. L. Smith & D. Boulter. 1983. Reproductive physiology of *Vicia faba* L. Pp. 133–142 *in* P. D. Hebblethwaite (editor), The Faba Bean (*Vicia faba* L.). Butterworths, London.

Ghosh, S. & K R. Shivanna. 1982. Anatomical and cytochemical studies on the stigma and style in some legumes. Bot. Gaz. (Crawfordsville) 143: 311–318.

Gunn, C. R. & J. Kluve. 1976. Androecium and pistil characters for the tribe *Vicieae* (Fabaceae). Taxon 25: 563–575.

Halterlein, A. J., C. D. Clayberg & I. D. Teare. 1980. Influence of high temperature on pollen grain viability and pollen tube growth in the styles of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci. 105: 12–14.

Hanson, C. H. 1953. *Lespedeza stipulata:* stigma morphology, meiosis, microgametogensis, and fertilization. Agron. J. 45: 200–203.

——— & W. A. Cope. 1955. Reproduction in the cleistogamous flowers of ten perennial species of *Lespedeza*. Amer. J. Bot. 42: 624–627.

Hawkins, C. F. & A. M. Evans. 1973. Elucidating the behaviour of pollen tubes in intra- and inter-specific pollinations of *Phaseolus vulgaris* L. and *P. coccineus* Lam. Euphytica 22: 378–385.

Heslop-Harrison, J. & Y. Heslop-Harrison. 1982a. Pollen–stigma interaction in the Leguminosae: constituents of the stylar fluid and stigma secretion of *Trifolium pratense* L. Ann. Bot. (London) 49: 729–735.

——— & ———. 1982b. Pollen–stigma interaction in the Leguminosae: The secretory system of the style in *Trifolium pratense* L. Ann. Bot. (London) 50: 635–645.

——— & ———. 1983. Pollen–stigma interaction in the Leguminosae: The organization of the stigma in *Trifolium pratense* L. Ann. Bot. (London) 51: 571–583.

——— & ———. 1984. Stigma organization and the control of fertilization in *Phaseolus*. Pp. 88–96 *in* R. Reimann-Philipp (editor), Proceedings of the Eucarpia meeting on *Phaseolus* bean breeding, Hamburg, July 1983.

Heslop-Harrison, Y. & K. R. Shivanna. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. (London) 41: 1233–1258.

Holden, J. H. W. & D. A. Bond. 1960. Studies on the breeding system of the field bean (*Vicia faba* Linn.). Heredity 15: 175–192.

Jost, L. 1907. Über die selb-sterilität einiger Blüten. Bot. Zeitung, 2, Abt. 65: 77–117.

Kambal, A. E., D. A. Bond & G. Toynbee-Clarke. 1976. A study of the pollination mechanism in field beans (*Vicia faba* L.). J. Agric. Sci. 87: 519–526.

Kreitner, G. L. & E. L. Sorensen. 1984. Stigma development and the stigmatic cuticle of alfalfa, *Medicago sativa* L. Bot. Gaz. (Crawfordsville) 145: 436–443.

_____ & _____. 1985. Stigma development and the stigmatic cuticle of *Medicago scutellata*. Canad. J. Bot. 63: 813–818.

Lackey, J. A. 1979. Sketches on flower dissections in Phaseolus (Fabaceae) Faboideae. Isleya 1: 19–54.

_____. 1980 Sketches of flower dissections in American Phaseolinae (Leguminosae—Papilionoideae). Isleya 1: 167–186.

_____. 1981. Phaseoleae. Pp. 301–321 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____. 1983. Review of generic concepts in American Phaseolinae (Fabaceae) Faboideae. Isleya 2: 21–64.

Lakshmi, K. V. 1984. Structure and Cytochemistry of the Pistil in Some Legumes. M. Phil. Thesis. University of Delhi.

_____ & K. R. Shivanna. 1985. Structure and cytochemistry of the pistil in *Arachis hypogaea*. Proc. Indian Acad. Sci. (Pl. Sci.) 95: 357–363.

Lord, E. M. 1981. Cleistogamy: a tool for the study of floral morphogenesis, function and evolution. Bot. Rev. (Lancaster) 47: 421–449.

_____ & Y. Heslop-Harrison. 1984. Pollen–stigma interaction in the Leguminosae: Stigma organization and the breeding system in *Vicia faba* L. Ann. Bot. (London) 54: 827–836.

_____ & L. V. Kohorn. 1986. Gynoecial development, pollination, and the path of pollen tube growth in the tepary bean, *Phaseolus acutifolius*. Amer. J. Bot. 73: 70–78.

_____ & B. D. Webster. 1979. The stigmatic exudate of *Phaseolus vulgaris* L. Bot. Gaz. (Crawfordsville) 140: 266–271.

Malti & K. R. Shivanna. 1983. Pollen–pistil interaction in chickpea. Int. Chickpea Newslett. 9: 10–11.

_____ & _____. 1984. Structure and cytochemistry of the pistil in *Crotolaria retusa* L. Proc. Indian Acad. Sci. 50 B: 92–102.

_____ & _____. 1985. The role of the pistil in screening compatible pollen. Theor. Appl. Genet. 70: 684–686.

Marcellos, H. & T. Perryman. 1986. Pollination and fertilization in *Vicia faba* L. Pp. 79–85 *in* R. B. Knox & E. Williams (editors), Pollination '86. University of Melbourne.

Mattsson, O., R. B. Knox, J. Heslop-Harrison & Y. Heslop-Harrison. 1974. Protein pellicle as a probable recognition site in incompatibility reactions. Nature 213: 703–704.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants. Agricultural Handbook No. 496, Agricultural Research Service, United States Department of Agriculture.

Mulcahy, D. L. 1979. The rise of angiosperms: a genecological factor. Science 206: 20–23.

_____. 1984. Manipulation of gametophytic populations. Pp. 114–119 *in* W. Lange, A. C. Zeven & N. C. Hogenboom (editors), Efficiency in Plant Breeding. PUDOC, Wageningen.

Newell, C. R. & T. Hymowitz. 1983. Hybridization in the genus *Glycine* Willd. (Leguminosae, Papilionoideae). Amer. J. Bot. 70: 334–348.

Ojehomon, O. O. 1972. Fruit abscission in cowpea *Vigna unguiculata* (L.) Walp. I. Distribution of 14C-assimilates in the inflorescence, and comparative growth of ovaries from persisting and abscising open flowers. J. Exp. Bot. 23: 751–761.

_____. 1975. Fruit abscission in cowpea *Vigna unguiculata* (L.) Walp. II. Quantitative estimations of total nucleic acids in ovaries of persisting and abscissing flowers. J. Exp. Bot. 26: 921–926.

Owens, S. J. 1985. Seed set in *Lotus berthelotii* Masferrer. Ann. Bot. (London) 55: 811–814.

Paul, C., P. Gates, N. Harris & D. Boulter. 1978. Asynchronous sexual development determines the breeding systems in field beans. Nature 275: 54–55.

Pazy, B. 1984. Insect Induced Self-pollination. Pl. Syst. & Evol. 144: 315–320.

Polhill, R. M. 1981. Papilionoideae. Pp. 191–205 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____, P. H. Raven & C. H. Stirton. 1981. Evolution and Systematics of Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Sacher, R. F., D. L. Mulcahy & R. C. Staples. 1983. Developmental selection for salt tolerance during self-pollination of *Lycopersicon* × *Solanum* F1 for salt tolerance of F2. Pp. 329–334 *in* D. L. Mulcahy & E. Ottaviano (editors), Pollen: Biology and Implications for Plant Breeding. Elsevier Biomedical, New York, Amsterdam, Oxford.

Sangduen, N., E. L. Sorensen & G. A. Liang. 1983. Pollen germination and pollen tube growth following self-pollination and intra- and inter-specific pollination of *Medicago* species. Euphytica 32: 527–534.

Sastri, D. C. 1984. Incompatibility in angiosperms: significance in crop improvement. Advances Appl. Biol. 10: 71–111.

_____ & J. P. Moss. 1982. Effects of growth regulators on incompatible crosses in the genus *Arachis* L. J. Exp. Bot. 33: 1293–1301.

Savithri, K. S., P. S. Ganapathy & S. K. Sinha. 1980. Sensitivity to low temperature in pollen germination and fruit-set in *Cicer arietinum* L. J. Exp. Bot. 31: 475–481.

Shivanna, K. R. & B. M. Johri. 1985. The Angiosperm Pollen: Structure and Function. Wiley Eastern Limited, New Delhi.

Singh, D. P. 1981. Breeding for resistance to diseases in green gram and black gram. Theor. Appl. Genet. 59: 1–10.

Sinha, S. K. 1977. Food Legumes: Distribution, Adaptability and Biology of Yield. Food and Agricultural Organization, Rome.

Small, E. & B. S. Brookes. 1983. The systematic value of stigma morphology in the legume Trifolieae with particular reference to *Medicago*. Canad. J. Bot. 61: 2388–2404.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. & Syst. 12: 253–279.

Tilton, V. R., L. W. Wilcox, R. G. Palmer & M. C. Albertsen. 1984. Stigma, style and obturator of soybean, *Glycine max* (L.) Merr. (Leguminosae) and their function in the reproductive process. Amer. J. Bot. 71: 676–686.

Townsend, C. E. 1971. Advances in the study of incompatibility. Pp. 281–309 *in* J. Heslop-Harrison (editor), Pollen: Development and Physiology. Butterworths, London.

Toynbee-Clarke, G. 1971. Pollination studies with highly inbred lines of winter beans (*Vicia faba* L.). J. Agric. Sci. 77: 213–217.

_____. 1974. The response to various pollination treatments in inbred lines of horse and tick beans (*Vicia faba* L.). J. Agric. Sci. 82: 531–534.

Turano, M. J., L. M. Baird & B. D. Webster. 1983. Characteristics of the stigma of chickpea. Crop Sci. 23: 1033–1036.

Van Stevenick, R. F. M. 1957. Factors affecting the abscission of reproductive organs in yellow lupins (*Lupinus luteus* L.). I. The effect of different patterns of flower removal. J. Exp. Bot. 8: 373–381.

Webster, B. D., S. P. Lynch & C. L. Tucker. 1979. A morphological study of the development of reproductive structures of *Phaseolus lunatus* L. J. Amer. Soc. Hort. Sci. 104: 240–243.

_____, C. L. Tucker & S. P. Lynch. 1977. A morphological study of the development of reproductive structures of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci. 102: 640–643.

Whealer, W. A. & D. D. Hill. 1957. Grassland Seeds. Van Norstrand Company, Princeton.

Williams, W. 1947a. Genetics of red clover (*Trifolium pratense* L.) compatibility. II (a) Homozygous self-sterile Sx Sx genotypes obtained as a result of pseudo-fertility; (b) self-fertility. J. Genet. 48: 51–68.

_____. 1947b. Genetics of red clover (*Trifolium pratense* L.) compatibility III. The frequency of incompatibility S-alleles in two non-pedigree populations of red clover. J. Genet. 48: 69–79.

Williams, R. D. & R. A. Silow. 1933. Genetics of red clover (*Trifolium pratense* L.) compatibility. I. J. Genet. 27: 341–361.

Zamir, D., S. D. Tanksley & R. A. Jones. 1981. Low temperature effect on selective fertilization by pollen mixtures of wild and cultivated tomato species. Theor. Appl. Genet. 59: 235–238.

A MULTIDISCIPLINARY APPROACH TO POLLINATION BIOLOGY IN THE LEGUMINOSAE

B. D. Schrire[1]

Abstract

One of the recurring problems in pollination biology is how to deal with the complexity of the subject. There is a need for a guide as to what to observe and record, thus enabling researchers to deal with a broad range of aspects of pollination. It is suggested that a framework of guidelines be used that summarizes a wide variety of accepted ideas needing to be tested in particular taxa to see how or if they apply. A selection of examples is used to illustrate how these guidelines have been applied to studies of pollination biology in legumes. The ultimate form of the flower, and its role in pollination, is a compromise between many internal and external constraints acting on it through evolutionary time. For a particular taxon, these constraints emerge as a unique set of physical, ecological, and chemical limitations that influence adaptation by natural selection. In the flower, these constraints are imposed by genetic influences, phylogeny, development, architecture, and by the physiological requirements of homeostasis, reproduction, deterrent defenses, and attraction mechanisms. This model of the flower requires a reappraisal of the many single characters traditionally used in systematics. Such characters may be compromised through being involved in more than one function. The aim of re-evaluating these characters is to suggest that they become more meaningful if linked to function. This will draw attention to characters and character relationships that might otherwise not be obvious. Central to this model is the concept of the functional complex. This is defined as a set of functional characters that interact as a relatively discrete and autonomous unit at least at some period during the life cycle of the plant. Within the pollination functional complex, for example, characters expressing particular functional traits may comprise one or a number of closely linked morphological, ecological, or chemical characters. These functional traits are called biological characters (function related either to a structure, process, or behavior). Identifying them is considered important because their function is the clue to understanding the relationships within and evolutionary development of that functional complex. A variety of constraints, however, impose restrictions on the number, type, and expression of biological characters within each functional complex. The ultimate aims of this approach are to encourage pollination biologists to provide greater comparability of results and uniformity of structure in their studies, and to stimulate systematists to interpret biological characters in the light of: a) the number and type of constraints giving rise to such characters in the broad group of the taxa under study; b) the sequence in which major constraints became canalized; and c) the pattern of biological characters that determines the uniqueness of each taxon.

I. Introduction

Cruden (in Baker & Hurd, 1968) summarized a number of topics which he felt should be covered in any pollination study: 1) identification of plants and flower visitors; 2) attraction devices; 3) breeding systems and floral behavior; 4) collection and study of flower visitors throughout the distribution range of the plants; 5) detailed observations on behavior of flower visitors; 6) study of pollinators for daily or seasonal cycles or periodicities; 7) collection of flower visitors from other plant species in the habitat of the study plant; and 8) analysis of pollen carried by flower visitors. Cruden stressed the error of believing that such studies are either simple or easy, although initially many of the relationships might appear to be obvious and easily discovered. He concluded that such a broad view was essential to understanding the basic relationships of ecology and evolution of both

[1]Botanical Research Institute, Private Bag X101, Pretoria, 0001 South Africa.

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plants and their floral visitors. This idea was extended by Macior (1971), who felt that too few aspects of the dynamics of the pollination mechanism were being addressed or reported. What was needed was an integrated approach that involved multidisciplinary studies.

One of the recurring problems in pollination biology is how to deal with the complexity of the subject. There is a real need to expand the overall approach suggested by Cruden and Macior, and in this paper a framework of guidelines for research, drawn from a number of disciplines, is suggested for application in studies of the Leguminosae.

Much is already known about the pollination biology of Leguminosae, but as Arroyo (1981) indicated, the data is too narrowly drawn to enable a really detailed synthesis. This literature analysis, which places more emphasis on areas she did not cover in much detail (e.g., melittophily and mechanisms in the Papilionoideae) confirms that the situation is largely unchanged. For this reason, it was decided to draw together, in an accessible way and referenced by selected examples, the various aspects of pollination biology in the Leguminosae that might be important in developing an overall synthesis later. In selecting examples, cases have been chosen that best illustrate a particular concept. It has not been the intention to critically review the literature.

The two major aims of this paper are first to encourage other systematists to broaden the perspective of their studies, for example by using pollination data, and second to provide pollination biologists with an idea of the type of characters which could be useful to systematists.

As a user of pollination data, it was found that the contents of many pollination studies are rarely comparable. Clearly, a more uniform approach would be desirable, and it is hoped that the framework of guidelines will provide the necessary ideas for a more uniform structure in pollination studies.

II. The Approach

The appendix comprises a series of guidelines for research that cover a comprehensive range of aspects dealing with pollination, grouped under several major headings. The points under these major headings should be applied to particular taxa to ensure that a broad base of information is used for the study, and they either require a direct observation or the testing of relationships. They are a guide to the researcher as to what to observe and analyze both in the field and in the laboratory, and they need to be rigorously tested in the subject taxa to see how or if they apply.

A selection of examples is used to illustrate how some of these guidelines have been applied to studies of pollination biology in the Leguminosae, but as is stressed again, it is not an exhaustive survey of the published evidence. The following section outlines a constraints model of the flower, which may be helpful to systematists in interpreting which of the points in the guidelines are particularly relevant to the taxa under study.

CONSTRAINTS MODEL

Much of the way that morphological characters tend to be analyzed by systematists stems from undue emphasis on what Gould & Lewontin (1979) called the adaptationist program. This regards natural selection as so powerful that the direct production of adaptation through its operation becomes the primary cause of nearly all organic form, function, and behavior.

Organisms are broken up into unitary "traits," and these are explained as being optimally designed by natural selection for their functions. An adaptive story is proposed for each, considered separately.

Traditionally, plant systematists think of flowers as static combinations of "primitive" and

"derived" morphological characters, chiefly there for the purpose of attracting the attention of pollinators to bring about fertilization; that is, the concept of bee, bird, or butterfly flowers (Leppik, 1966). Richards (1986), however, warned of the dangerous tendency to overgeneralize syndromes of flowers that are associated with particular visitors.

It is through flowers that plants reproduce (derived agamosperms excepted); therefore, selective forces for evolution must act strongly on floral form to optimize the reproductive potential of the plant (Kevan, 1984). The major trends in floral specialization, that is, primitive to derived characters, should be looked at in combination with one another and particularly in terms of biological "needs" of the life history.

Developing this concept, flowers can be seen as evolutionary compromises, best satisfying a number of selective pressures such as the: 1) provision of gametogenesis; 2) repulsion of mammalian, avian, and insect herbivores and resistance to attack by microorganisms; 3) attraction of suitable pollinators; 4) warding off, discouragement, or confusion of unwanted opportunists and robbers; 5) optimization of the traffic of pollen to a suitable receptive stigma; 6) possible selection of potential zygotes; 7) provision for embryogenesis and adequate protection of developing seeds; 8) determination of the type of propagule and provision of an effective mode for dispersal; and 9) provision for autonomous needs such as maintenance, repair, and growth.

Some or all of these demands can be met in any number of ways in time, space, behaviorly, or structurally, provided the components interact in a way that is not deleterious to the whole. This is illustrated in the apparently uniform structure of the papilionoid flower, which nevertheless gives rise to many distinctive floral forms that are a variation on a theme.

Flowers are being interpreted here as best compromises among competing demands, where each part cannot be optimized alone without imposing expenses on others. However, this idea of tradeoffs among competing selective demands (Calow, 1982) again emphasizes natural selection as the only cause of structure and function. Rather, organisms are integrated entities and not collections of discrete objects. Optimization frequently is not achieved, nor is adaptation unrestrained. This is due to various constraints imposed on the body plan of the organism (Gould & Lewontin, 1979; Calow, 1982; Webb, 1984), and natural selection may only be operating within the bounds of such constraints. These constraints are genetic, phylogenetic, developmental, architectural, and physiological. Genetic constraints are limits on genetic variation that are unlikely to be affected by natural selection, for example, limits imposed by genetic drift or molecular drive (concepts discussed in Dodson & Dodson, 1985). Phylogenetic constraints include the ways in which the evolutionary history of a taxon influences developmental pathways. Phylogeny is not under similar constraints throughout its duration. It is interrupted by periods when the number and type of constraints change. If the interaction of constraints is intense, then canalization will occur, limiting the direction of further change. However, a weakening or change in constraints can lead to new developmental lines being generated.

As components become constrained and canalized, adaptation is likely to be more parsimonious. This resistance, due to the accumulative effect of constraints, gives rise to the process of systemization in the sense of Riedl (1978), defined as a systematic reduction in the number of possible "decisions" that can be made as a line becomes canalized. However, a relaxation in constraints might lead to a repatterning of characters from a previous line and the development of a range of new compromises with different adaptive properties (the epigenetic landscape of Waddington, 1975).

With shifts in constraints, there may be a transfer of function to alternative structures, leaving the original structure to either assume a different function or become vestigial. Major shifts in constraints have characterized the development of related higher order taxa such as families, tribes, and genera, whereas minor constraint shuffles within the domain of previous canalization give rise to related species (see Tucker, this volume).

Developmental constraints (Gould & Lewontin, 1979; Alberch, 1982; Webb, 1984) include limits acting on the developmental pathway through ontogenetic time; that is, conservation of organiza-

tion, parsimony, timing of initiation, timing of maturation, and allometry. Structures formed by a complex sequence of developmental processes tend to be conserved over long periods of time; for example, the recurring tendency to modify existing structures rather than create new ones *ex nihilo* (Gould & Lewontin, 1979). This is an illustration of the principle of conservation of organization (Stebbins, 1973).

Adaptive modification tends to occur along the lines of least resistance (Ganong, 1901; Stebbins, 1950) leading to developmental canalization. Waddington (1975) refers to the change from one structure into another as a homoeitic transformation and the mechanisms underlying them as developmental canalization.

Constraints that distinguish major suprageneric levels in the Leguminosae (such as floral symmetry, order of initiation of floral parts, number and arrangement of parts, fusion, and loss of parts) are manifested early in ontogeny and are relatively stable (Tucker, 1984, this volume). Some characteristics manifested during mid development of flowers (during enlargement and shape changes) likewise tend to be stable and to characterize tribes and genera in the Leguminosae. Characteristics separating related species tend to be manifested late in floral development during the stage of cell differentiation (Tucker, 1984) and are more labile than those occurring earlier in floral ontogeny. Therefore, the earlier that constraints arise in ontogeny, the more stable they are and the greater effect they have on the system. This can also relate back to the level at which the constraints arose in the taxonomic hierarchy. Determining the ontogenetic bases for floral characteristics can distinguish cases of homology from those of convergence and can clarify relationships among related species (Humphries & Funk, 1984; Tucker, 1984).

Alterations in the timing of maturation (heterochrony) may lead to developmental amplification (Gould, 1977; Calow, 1983). This involves alteration in the rate and timing of growth (increase in spatial dimensions and weight), development (morphogenesis, differentiation, and increase in complexity of organization), and maturation (cessation of development and growth and the onset of the reproductive processes). Development can be broadly decelerated or truncated relative to the ancestors so that maturity occurs at an earlier developmental stage (paedomorphosis), for example, the processes of neoteny (retardation) and progenesis; or broadly accelerated relative to the ancestor so maturity occurs at a later developmental stage, for example, hypermorphosis (Calow, 1983).

Allometric growth may also occur, where the form of a part is a correlated consequence of selection directed elsewhere (discussed in Gould & Lewontin, 1979; Dodson & Dodson, 1985).

Architectural constraints (Gould & Lewontin, 1979) include aspects of design, dimensions, and materials that limit the array of possible structures of an organ (constructional compromise). The structures of parts of a flower are the result of phylogeny, development, and architecture, but their present adaptation may be a secondary phenomenon representing an incidental use of available parts by selection. The architectural constraint is primary, and the fact that a structure is used in some way should not be confused with the primary evolutionary reason for its existence and conformation (Gould & Lewontin, 1979). In addition, many so-called "vestigial characters" may either have a function earlier in ontogeny or may be a consequence of previous architectural constraints.

Physiological constraints may be physical or chemical, and include those limits placed on the organism by the requirements for homeostasis (such as maintenance, growth, and repair), reproduction, deterrent-defenses, and attraction mechanisms. Such constraints have a bearing on the energetics of the organism.

Organisms in fact may be so constrained by genetic influences, phyletic heritage, pathways of development, and general architecture that the constraints themselves may be more important in delimiting pathways of change than is the selective force that mediates change when it occurs. This is not to say, however, that most constraints are without a selective origin. Many characters arise through natural selection that will limit and channel further adaptation in certain directions if they become successful and fixed in the taxon (Riedl, 1978).

FUNCTIONAL COMPLEXES

Central to the constraints model is the concept of a functional complex. A functional complex may be defined as a set of functional characters that interact as a relatively discrete and independent unit at least during some period of the life cycle of the organism. Entire complexes of characters contribute towards specific functions within an organism (Cain & Harrison, 1958; Cain, 1959). Adey (1982) has provided evidence for the existence of functional complexes of characters, within each of which cohesion is greater and between which congruence is less than for randomly selected character sets.

If different taxa are compared and defined using sets of characters from the same functional complex, they are more likely to show congruence than if the character sets were chosen from different functional complexes. This follows from the principle of mosaic evolution (Stebbins, 1984), where constancy with respect to one set of characters will be accompanied over a given period of time by a more or less rapid change with respect to other characteristics of the same organism. To the extent that the characters involved are functional and adaptive and are subject to different evolutionary forces, mosaic evolution must be guided by mosaic selection. Stebbins (1984) noted that the primitive or derived nature of individual character states cannot be deduced solely on the basis of their correlation or association with other character states. Stevens (1986) raises the same problem with respect to the apparently similar concept of heterobathmy or "discordant" character change. Here, greater change may occur in one lineage than in another, or only some organs may change in a lineage.

Functional complexes can be envisaged as a hierarchy extending from functions at a cellular level, or below, to a level comprising all the functions of the organism (Adey, 1982). The hierarchy is constructed so that members of a functionally defined character set are more functionally related to each other than to members of other functional groups. The level of designation of the functional complex is arbitrary and depends on the aims of the research.

It might seem self-evident that the parts of a flower are linked anatomically, physiologically, and phylogenetically into a functioning organ, but when viewed as a series of functional complexes, it is the different permutations of characters in different taxa that become significant. Within this context, the present study focuses on the pollination functional complex in legume flowers. This impinges to a greater or lesser extent on five related functional complexes: floral morphogenesis, gametogenesis and gametophyte development, embryogenesis, fruit and seed development, and fruit and seed dispersal.

BIOLOGICAL CHARACTERS

Many of the traditional morphological characters used in taxonomic investigations, such as length of calyx, size of petals, degree of stamen fusion, type of stigma, or number of ovules, are revealed as being parts of closely linked character complexes. For example, the design, arrangement and degree of fusion of floral parts, the presence or absence of nectar, the breeding system, and type of pollinator are all integral to the tripping mechanism in papilionoid flowers.

The tripping mechanism increases the efficiency of pollen transfer, protects resources from opportunists and herbivores, selectively attracts and rewards suitable pollinators, and allows the maintenance of self-compatibility while finely controlling levels of outbreeding (Arroyo, 1981). As can be seen, the tripping mechanism has many functions, and how it increases the efficiency of pollen transfer, for example, is only one of these. The efficiency of pollen transfer expresses a particular functional trait (function related to process) and may comprise one or a number of closely linked morphological, ecological, or chemical characters. In *Pseudarthria hookeri*, for example, the

pollen is relatively dry and is directed over the undersurface of the bee by the explosive tripping mechanism of the flower. The accuracy of pollen expulsion ensures little wastage and makes for a highly efficient transfer of an energetically expensive resource (Schrire, 1984). Such a functional trait (i.e., function related either to a structure, process, or behavior) is called a biological character. For tripping mechanisms to be homologous when compared in two species, all aspects of the mechanism must be congruent.

Another example where traits may be deceptively similar is in petal coloration. Consider two species having blue flowers. Unless one can show that flower color is produced similarly, one may not be able to say that color in the two species is homologous. This is because color can be produced physically (by diffraction and refraction) or chemically (by pigmentation). The color may be due to either of these or a combination of both. It is possible also that different surface micromorphologies will affect the type of structural color produced, and similarly the localization, biosynthesis, and type of pigments will affect chemical coloration. So flower color, to be really comparable, needs to be considered in terms of the surface micromorphology and the type and localization of the pigments involved. The functional role of the colors may be different, for example, as different recognition cues for pollinators, or they may be similar but nonhomologous due to convergence. In either case, identification of the biological character color (function related to structure or process) will reveal nonhomologous traits and produce "purer" characters with a lower level of homoplasy.

The identification of biological characters is also considered important because their function is the clue to understanding the relationships within and evolutionary development of that functional complex. The problem with interpreting many morphological characters is that they usually have a role in different functions. They are therefore push–pull compromises, being the result of what best suits the role they play in each different function.

The role of each biological character may also change over time. A calyx may be protective in the bud stage, both physically and chemically by glandular exudates, but it may play an additional structural-supportive role for pollinator landing while the flower is open. It may also take over the attractive role from petals, thus acting as an orientation cue to pollinators. It can also serve to protect the floral nectary, act as a receptacle for nectar accumulation, or support extrafloral nectaries. In fruit the calyx may become accrescent and either protect or aid in seed dispersal. It may therefore also participate in a number of different functional complexes.

The various types of constraints were discussed above, but how are they manifested? Constraints, as used in this paper, are limiting features which impose a restriction on the number and type of biological characters that can be successfully expressed in the pollination functional complex. Constraints, therefore, refer to those features in a functional complex that circumscribe one or a number of related biological characters. For example, particular tripping mechanisms in papilionoid flowers circumscribe a number of related biological characters associated with that tripping mechanism. An explosive method of pollen release imposes, among others, the following restrictions on the flower: a certain conformation of floral parts; a particular pattern of rewards (nectaries tend to be lost and pollen becomes the major reward); and flowers that can only be tripped once, therefore opening for only one day. These all influence both the type and foraging behavior of pollinators. A pump method of pollen release imposes a different conformation on the floral parts, and flowers can be visited many times (the rewards need to be available for long periods; hence nectar is offered instead of pollen). This pattern elicits a different foraging behavior from pollinators than above. Each tripping mechanism also circumscribes particular attraction or defense devices, certain methods of transferring pollen to the vector and female gametophyte, certain aspects of the breeding system such as the degree of self-compatibility, and particular phenological patterns such as the length of the flowering period.

The general pattern of constraints, by influencing the expression of biological characters, should also indicate whether homoplasy (convergence, parallelism, etc.) is involved when taxa are compared.

III. Using the Guidelines (see Appendix)

The framework of guidelines deals with the pollination functional complex in legume flowers. The way in which systematists use the guidelines may well differ from that of pollination biologists. Systematists adopt a phylogenetic perspective when interpreting pollination data, whereas for pollination biologists, most of whom work at the infrageneric level, this is not essential. Nevertheless, it is hoped that pollination biologists will use these guidelines to broaden the base of their studies and to provide comparable data in a more unified way. It is suggested that plant systematists might use the following process to interpret data from pollination studies.

In the group of taxa being studied, use the guidelines to find which aspects are acting as: 1) constraints or limiting features chaneling variation in the pollination functional complex; 2) biological characters (i.e., function must be clearly shown to be related to either a structure, process, or behavior). If characters are multifunctional and function cannot be determined precisely, or if the function is unknown, then the structure, process, or behavior should be used with care and in combination with the above only if it is considered taxonomically important. Constraints may be manifested by whether characters are fixed or labile. The more intense the constraints, the more fixed characters are likely to be. In trying to identify either constraints or biological characters, it may help to look at various isolating mechanisms between species.

Interpret the biological characters in the light of: 1) the number and type of constraints giving rise to these characters in the broad group of the taxa under study; 2) the sequence in which major constraints became canalized (this can usually be inferred from how they are spread across the taxonomic hierarchy, that is, the degree of occurrence both within and between families, genera, and species); and 3) the pattern of characters that determines the uniqueness of each taxon.

IV. Conclusions

1. The pluralistic view of organisms proposed by Gould & Lewontin (1979) is upheld. This recognizes not only the importance of natural selection and adaptation in the evolution of organisms but also stresses the significance of constraints and modes of development.
2. Organisms are integrated wholes, but the recognition of hierarchies of functional complexes makes it possible to focus on subsystems and to study their complexity and interrelationships.
3. A developmental sequence is recognized within the flower as an organ, which can be expressed as a series of functional complexes, that is, floral morphogenesis, the processes of male and female gametogenesis, pollination, embryogenesis, fruit and seed development, and fruit and seed dispersal.
4. If different taxa are compared and defined using sets of characters from the same functional complex, they are more likely to show congruence than if the character sets were chosen from different functional complexes. This should avoid the apparently similar problems caused by mosaic evolution and heterobathmy.
5. Within each functional complex a number of constraints or limiting features impose restrictions on the number, type, and expression of various biological characters. The way constraints have developed is the process by which patterns of biological characters that define taxa are formed.
6. A framework of guidelines (see Appendix) has been compiled and covers a wide range of accepted ideas known to influence pollination biology. The guidelines should only be considered as pointers, however, requiring direct observation or the testing of relationships to see how or if they apply in the taxon (taxa) being studied. Depending on these observations, the results should be interpreted in terms of being either constraints or biological characters within the pollination functional complex.
7. The functionality of biological characters is a clue to how evolution has acted in the group, but function can change through time so that present function may be unrelated to original struc-

ture. It is important, therefore, to identify the constraints in the functional complex and to have an understanding of the sequence in which they arose.

8. Identification of biological characters (linking function to either a structure, process, or behavior) will improve the type of characters used, since they help clarify problems of homology and homoplasy. Evolutionary polarity will be easier to assign in such characters when considered in the context of the prevailing constraints.
9. The use of constraints and biological characters, selected from a number of functional complexes, is likely to improve the level of information and fine-tuning in empirical methods of study such as phenetics and cladistics. They could also be valuable in deciding which of a number of equally parsimonious trees is the best reflection of phylogeny.
10. The geographical distribution of biological characters might yield distinct patterns of correlation associated with different regions. The mapping of such characters should provide important insights into plant species and their functional relationship to the biota they encountered as they dispersed and evolved.
11. The search for constraints and biological characters within a functional complex broadens the base and type of data gathered for studies of complex natural systems.
12. Constraints and biological characters should also be identified to explore relationships that are not immediately apparent. That is, characters may be linked in time or space to other components in the system. Interaction makes the whole greater than the sum of its parts.
13. The potential of particular biological characters (functional traits) in wild species could be selected for the breeding of related economically important plants (see Plitmann & Kislev, this volume).
14. Pollination biologists may see this approach as a call by plant systematists for greater comparability of data and uniformity of structure in pollination studies. Systematists, in turn, could synthesize this biological information to hopefully reflect both phylogeny and phytogeography, thereby leading toward a better understanding of plant systematics.

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References

Adey, M. E. 1978. Pollination: The gorses and brooms pollinator survey. Bot. Surv. Brit. Isles Newslett. 20: 7–8.

———. 1982. Taxonomic aspects of plant–pollinator relationships in the Genistinae (Leguminosae). Ph.D thesis. University of Southampton, United Kingdom.

Agren, G. I. & T. Fagerstrom. 1980. Increased or decreased separation of flowering times? The joint effect of competition for space and pollination in plants. Oikos 35: 161–164.

Akerberg, E. 1967. Pollination problems in some melliferous fodder legumes. XXI Int. Beekeep. Congr. Prelim. Sci. Meeting Summ. Pap. 29: 64–65.

Alberch, P. 1982. Developmental constraints in evolutionary processes. *In:* J. T. Bonner (editor), Evolution and Development. Springer-Verlag, Berlin.

Allen, O. N. & E. K. Allen. 1981. The Leguminosae: A Sourcebook of Characteristics, Uses and Nodulation. Macmillan, London & Basingstoke.

Ancibor, E. 1969. Los nectarios florales en Leguminosas–Mimosoideas. Darwiniana 15: 128–142.

Applebaum, S. W. & Y. Birk. 1979. Saponins. Pp. 539–566 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Armstrong, J. A., J. M. Powell, & A. J. Richards (editors). 1982. Pollination and Evolution. Royal Botanic Gardens, Sydney.

Arroyo, M. T. K. 1976. Geitonogamy in animal pollinated tropical angiosperms. A stimulus for the evolution of self-compatibility. Taxon 25: 543–548.

_____. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Augspurger, C. K. 1983. Phenology, flowering synchrony, and fruit set in six neotropical shrubs. Biotropica 15: 257–267.

Baker, H. G. & I. Baker. 1975. Studies of nectar-constitution and plant–pollinator coevolution. Pp. 100–140 *in* L. E. Gilbert & P. H. Raven (editors), Coevolution of Animals and Plants. Univ. of Texas Press, Austin.

_____ & _____. 1986. The occurrence and significance of amino acids in floral nectar. Pl. Syst. Evol. 151: 175–186.

_____ & P. D. Hurd. 1968. Intra floral ecology. Annual Rev. Entomol. 13: 385–414.

_____, I. Baker & P. A. Opler. 1973. Stigmatic exudates and pollination. Pp. 47–60 *in* N. B. M. Brantjes & H. F. Linskens (editors), Pollination and Dispersal. Symposium, Department of Botany, Nijmegen.

Baker, I. & H. G. Baker. 1982. Some chemical constituents of floral nectars of *Erythrina* in relation to pollinators and systematics. Allertonia 3: 25–37.

Barth, F. G. 1985. Insects and Flowers: The Biology of a Partnership. Allen & Unwin, London. [Translated by M. A. Biederman-Thorson.]

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85–92.

_____. 1980. Evolution of dioecy in flowering plants. Annual Rev. Ecol. Syst. 11: 15–39.

_____. 1983. Patterns of flowering in tropical plants. Pp. 394–410 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

_____ & J. H. Beach. 1981. Evolution of sexual systems in flowering plants. Ann. Missouri Bot. Gard. 68: 254–274.

_____ & C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees; implications for the evolution of paternal and maternal reproductive patterns. Amer. J. Bot. 71: 736–751.

Bell, E. A. 1972. Toxic amino acids in the Leguminosae. Pp. 163–178 *in* J. B. Harborne (editor), Phytochemical Ecology. Academic Press, London.

_____. 1981. Non-protein amino acids in the Leguminosae. Pp. 489–499 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Bentley, B. L. 1976. Extrafloral nectaries and protection by pugnacious bodyguards. Annual Rev. Ecol. Syst. 8: 407–427.

_____ & T. S. Elias (editors). 1983. The Biology of Nectaries. Columbia Univ. Press, New York.

Beresford, G. & R. Marginson. 1983. Birds and pollination in *Acacia terminalis*. Bull. Groupe Int. Étude Mimos. 11: 47–49.

Bernhardt, P. 1983. Insect pollination in Australian *Acacia*. Pp. 84–97 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors), Pollination '82. School of Botany, University of Melbourne.

_____, J. Kenrick & R. B. Knox. 1984. Pollination biology and the breeding system of *Acacia retinodes* (Leguminosae: Mimosoideae). Ann. Missouri Bot. Gard. 71: 17–29.

Bhattacharyya, B. & J. K. Maheshwari. 1971. Studies on extrafloral nectaries of the Leguminales 1: Papilionaceae. Proc. Indian Acad. Sci. 37: 11–30.

Bisby, F. A. 1981. Genisteae. Pp. 409–425 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Boardley, M., C. H. Stirton, & J. B. Harborne. 1986. A chemotaxonomic survey of the tribe Psoraleeae in Africa. Biochem. Syst. Evol. 14: 603–613.

Bohart, G. E. 1960. Insect pollination of forage legumes. Bee World 41: 57–64, 85–97.

Bond, D. A. & M. H. Poulsen. 1983. Pollination. Pp. 77–101 *in* P. D. Hebblethwaite (editor), The Faba Bean (*Vicia faba* L.). Butterworths, London.

Borchert, R. 1983. Phenology and control of flowering in tropical trees. Biotropica 15: 81–89.

Bos, J. J. 1967. The genus *Liparia* L. (Pap.). J. S. African Bot. 33: 269–292.

Bose, K., G. Tripathy, & R. D. Joshi. 1983. Some floral abnormalities in *Cassia auriculata* L. and *C. occidentalis* L. Legume Res. 6: 101–102.

Boughton, V. H. 1985. Extrafloral nectaries of some Australian bipinnate acacias. Austral. J. Bot. 33: 175–184.

Buchmann, S. L. 1974. Buzz pollination of *Cassia quiedondilla* (Leguminosae) by bees of the genera *Centris* and *Melipona*. Bull. S. Calif. Acad. Sci. 73: 171–173.

———. 1983. Buzz pollination in angiosperms. Pp. 73–113 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Burquez, A. & K. J. Sarukhán. 1980. Biología de poblaciones silvestres y cultivados de *Phaseolus coccineus* L.: 1. Relaciones planta–polinizador. Bol. Soc. Bot. México 39: 5–24.

Buss, P. A. & N. R. Lersten. 1972. Crystals in tapetal cells of the Leguminosae. Bot. J. Linn. Soc. 65: 81–85.

Cain, A. J. 1959. Function and taxonomic importance. Pp. 5–19 *in* A. J. Cain (editor), Function and Taxonomic Importance. Publ. Syst. Assoc., No. 3.

———& G. A. Harrison. 1958. An analysis of the taxonomists judgement of affinity. Proc. Zool. Soc. London 131: 85–98.

Calow, P. 1982. Economics of ontogeny—adaptational aspects. Pp. 81–104 *in* B. Shorrocks (editor), Evolutionary Ecology. 23rd Symp., Brit. Ecol. Soc., Leeds. Blackwell Scientific Publications, Oxford.

———. 1983. Evolutionary Principles. Blackie, Glasgow & London.

Carlquist, S. 1974. Island Biology. Columbia Univ. Press, New York.

Carpenter, F. L. 1983. Pollination energetics in avian communities: simple concepts and complex realities. Pp. 215–234 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Chow, K. H. 1974. Floral biology and seed development in *Alysicarpus vaginalis* DC., *Desmodium heterophyllum* (Willd.) DC. and *Desmodium triflorum* (L.) DC. J. Singapore Natl. Acad. Sci. 4: 6–10.

——— & L. V. Crowder. 1974. Flowering behavior and seed development in four *Desmodium* spp. Agron. J. 66: 236–238.

Clinch, G. P., T. Palmer-Jones, & I. W. Forster. 1972. Effect on honey bees of nectar from the yellow Kowhai (*Sophora microphylla* Ait.). New Zealand J. Agric. Res. 15: 194–201.

Colin, L. J. & C. E. Jones. 1980. Pollen energetics and pollination modes. Amer. J. Bot. 67: 210–215.

Compton, S. G. & D. A. Jones. 1985. An investigation of the responses of herbivores to cyanogenesis in *Lotus corniculatus* L. Biol. J. Linn. Soc. 26: 21–38.

Conn, E. E. 1979. Cyanide and cyanogenic glycosides. Pp. 387–412 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Couderc, H. 1980. Biologie florale de quelques espèces du genre *Anthyllis* L. et notamment de l'*A. montana* L. Bull. Soc. Bot. France 127: 139–149.

——— & R. Gorenflot. 1978. Adaptation de la fleur entomophile de l'*Anthyllis vulneraria* L. à l'autogamie. Bull. Soc. Bot. France 125: 369–378.

Cowan, R. S. 1981. Swartzieae. Pp. 209–212 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & R. M. Polhill. 1981a. Detarieae. Pp. 117–134 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & ———. 1981b. Amherstieae. Pp. 135–142 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Crawford, T. J. & D. A. Jones. 1986. Variation in the colour of the keel petals in *Lotus corniculatus* L. 2. Clines in Yorkshire and adjacent counties. Watsonia 16: 15–19.

Crowder, C. A. 1982. Systematics and phylogeny of the herbaceous North American and inland Argentine Sophora (Fabaceae). Ph.D. thesis. Texas Tech University.

Cruden, R. W. 1977. Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

——— & S. M. Hermann-Parker. 1979. Butterfly pollination of *Caesalpinia pulcherrima* with observations on a psychophilous syndrome. J. Ecol. 67: 155–168.

——— & K. G. Jensen. 1979. Viscin threads, pollination efficiency and low pollen–ovule ratios. Amer. J. Bot. 66: 875–879.

Dafni, A. 1984. Mimicry and deception in pollination. Annual Rev. Ecol. Syst. 15: 259–278.

———. 1986. Floral mimicry-mutualism and unidirectional exploitation of insects by plants. Pp. 81–90 *in* B. Juniper & R. Southwood (editors), Insects and the Plant Surface. Arnold, London.

Dahlgren, R. 1963. Studies on *Aspalathus* and some related genera in South Africa. Opera Bot. 9: 81–97, 218–234.

Davis, T. A. W. & S. B. Evans. 1980. Irregular times of flowering of *Ononis reclinata* L. Watsonia 13: 125–126.

Delgado, S., A. O. & M. S. Sánchez. 1977. Biología floral del género *Cassia* en la región de los Tuxtlas, Veracruz. Bol. Soc. Bot. México 37: 5–52.

Dodson, E. O. & P. Dodson. 1985. Evolution: Process and Product, 3rd edition. Prindle, Weber & Schmidt, Boston.

Ducke, A. 1901. Beobachtungen uber Blutenbesuch, Erscheinungenseit. . . . der bei Para vorkommenden Bienen. Z. Syst. Hymen. Dipterologie 1: 25–32.

Duffey, S. S. 1986. Plant glandular trichomes: their partial role in defence against insects. Pp. 151–183 *in* B. Juniper & R. Southwood (editors), Insects and the Plant Surface. Arnold, London.

Dulberger, R. 1981. The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpiniaceae). Amer. J. Bot. 68: 1350–1360.

Dwyer, J. D. & Y. Y. Chen. 1971. Style length and 'delphy in the Fabaceae. Trans. Missouri Acad. Sci. 5: 79–84.

Elias, T. S. 1981. Mimosoideae. Pp. 143–152 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Erickson, E. & S. L. Buchmann. 1983. Electrostatics and pollination. Pp. 173–184 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Esau, K. 1965. Plant Anatomy, 2nd edition. Wiley, New York.

Esch, H. 1967. The evolution of bee language. Sci. Amer. 216: 96–104.

Evenson, W. E. 1983. Experimental studies of reproductive energy allocation in plants. Pp. 249–274 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Faegri, K. & L. van der Pijl. 1979. The Principles of Pollination Ecology, 3rd edition. Pergamon Press, Oxford.

Feeny, P. 1975. Biochemical coevolution between plants and their insect herbivores. Pp. 3–19 *in* L. E. Gilbert & P. H. Raven (editors), Coevolution of Animals and Plants. Texas Univ. Press, Austin.

Feinsinger, P., J. A. Wolfe & L. A. Swarm. 1982. Island ecology: reduced hummingbird diversity and the pollination biology of plants, Trinidad and Tobago, West Indies. Ecology 63: 494–506.

_____, Y. B. Linhart, L. A. Swarm, & J. A. Wolfe. 1979. Aspects of the pollination biology of three *Erythrina* species on Trinidad and Tobago. Ann. Missouri Bot. Gard. 66: 451–471.

Ferguson, I. K. 1984. Pollen morphology and biosystematics of the subfamily Papilionoideae (Leguminosae) Pp. 377–394 *in* W. F. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

_____ & J. J. Skvarla. 1982. Pollen morphology in relation to pollinators in Papilionoideae (Leguminosae). Bot. J. Linn. Soc. 84: 183–193.

Fontenelle, G. B. 1979. Contribuição ao estudo de biologia floral de *Cassia silvestris* Vell.: considerações anatômicas. Leandra 8–9: 49–84.

Ford, H. A. & N. Forde. 1976. Birds as possible pollinators of *Acacia pycnantha*. Austral. J. Bot. 24: 793–795.

Frankel, R. & E. Galun. 1977. Pollination Mechanisms, Reproduction Plant Breeding. Springer-Verlag, Berlin.

Frankie, G. W. & H. G. Baker. 1974. The importance of pollinator behavior in the reproductive biology of tropical trees. Anales Inst. Biol. Univ. Nac. Mexico 45: 1–10.

_____ & R. Coville. 1979. An experimental study on the foraging behavior of selected solitary bee species in Costa Rican dry forest (Hymenoptera: Apoidea). J. Kansas Entomol. Soc. 52: 591–602.

_____ & W. A. Haber. 1983. Why bees move among mass-flowering neotropical trees. Pp. 360–372 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

_____, H. G. Baker & P. A. Opler. 1974. Comparative phenological studies of trees in tropical wet and dry forests in the lowlands of Costa Rica. J. Ecol. 62: 881–919.

_____, P. A. Opler & K. S. Bawa. 1976. Foraging behavior of solitary bees: implications for outcrossing of a neotropical forest tree species. J. Ecol. 64: 1049–1057.

FURGULA, B., T. A. GOCHNAUR & F. G. HOLDAWAY. 1958. Constituent sugars of some northern legume nectars. Bee World 39: 203–205.

GADGIL, M. & O. T. SOLBRIG. 1972. The concept of r- and k-selection: evidence from wildflowers and some theoretical considerations. Amer. Naturalist 106: 14–31.

GANDERS, F. R. 1979. The biology of heterostyly. New Zealand J. Bot. 17: 607–635.

GANONG, W. F. 1901. The cardinal principles of morphology. Bot. Gaz. (Crawfordsville) 31: 426–434.

GARCÍA, M. U. 1975. Floral biology of *Amherstia*. Pterocarpus 1: 26–35.

GENTRY, A .H. 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6: 64–68.

———. 1978. Anti-pollinators for mass flowering plants? Biotropica 10: 68–69.

GILL, F. B. 1978. Proximate costs of competition for nectar. Amer. Zool. 18: 753–763.

GORDON-GRAY, K. D. & C. J. WARD. 1975. A contribution to the knowledge of floral variation in *Acacia karroo* in eastern South Africa. Boissiera 24: 279–284.

GORI, D. F. 1983. Post-pollination phenomena and adaptive floral changes. Pp. 31–49 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

GOULD, S. J. 1977. Ontogeny and Phylogeny. Harvard Univ. Press, Cambridge, Massachusetts.

——— & R. C. LEWONTIN. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *In:* The Evolution of Adaptation by Natural Selection. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 205: 581–598.

GRANBY, R. 1980. Revision of the genus *Coelidium* (Liparieae-Fabaceae). Opera Bot. 54: 3–47.

GRANT, V. 1981. Plant Speciation, 2nd edition. Columbia Univ. Press, New York.

GREEN, T. W. & G. E. BOHART. 1975. The pollination ecology of *Astragalus cibarius* and *Astragalus utahensis* (Leguminosae). Amer. J. Bot. 62: 379–386.

GUERRANT, E. O., JR. & P. L. FIEDLER. 1981. Flower defences against nectar-pilferage by ants. *In:* Reproductive Botany. Biotropica 13 (Suppl., No. 2): 25–33.

GULYAS, S. & I. KINCSEK. 1982. Floral nectaries of species of Papilionaceae. Acta Biol. (Szeged 1955+) 28: 53–63.

GUNN, C. R. & J. KLUVE. 1976. Androecium and pistil characters for tribe Vicieae (Fabaceae). Taxon 25: 563–575.

GUPTA, M. 1980. Trichomes occurring on floral parts in some Indian and African species of *Crotalaria*. Proc. Indian Acad. Sci. 89: 229–235.

HABER, W. A., G. W. FRANKIE, H. G. BAKER, I. BAKER & S. KOPTUR. 1981. Ants like flower nectar. Biotropica 13: 211–214.

HADDOCK, R. C. & S. J. CHAPLIN. 1982. Pollination and seed production in two phenologically divergent prairie legumes *(Baptisia leucophaea* and *B. leucantha)*. Amer. Midl. Naturalist 108: 175–186.

HANDEL, S. N. 1983. Pollination ecology, plant population structure, and gene flow. Pp. 163–211 *in* L. Real (editor), Pollination Biology. Academic Press, Orlando.

HARAGSIM, O. 1974. Nectar production of *Robinia pseudoacacia* in the lower Moldau area. Věd. Práce Výzk. Ústavu Včelářsk. v Dole u Libcic 6: 25–38.

——— & J. MACHA. 1969. Sugar contained in Japanese sophora *(Sophora japonica)*. Sborn. Českoslov. Akad. Zeměd. Věd, Rostl. Vyroba 15: 659–664.

HARBORNE, J. B. 1979. Flavonoid pigments. Pp. 619–655 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

———. 1982. Introduction to Ecological Biochemistry. Academic Press, London.

HARDIN, J. W., G. DOERKSEN, D. HERNDON, M. HOBSON & F. THOMAS. 1972. Pollination ecology and floral biology of four weedy genera in southern Oklahoma. Southw. Naturalist 16: 403–412.

HAWKESWOOD, T. J. 1985. The role of butterflies as pollinators of *Acacia bidwillii* Benth. (Mimosaceae) at Townsville, northern Queensland. Austral. J. Bot. 33: 167–173.

HEDSTROM, I. & M. THULIN. 1986. Pollination by a hugging mechanism in *Vigna vexillata* (Leguminosae–Papilionoideae). Pl. Syst. Evol. 154: 275–283.

HEINRICH, B. 1975. Energetics of pollination. Annual Rev. Ecol. Syst. 6: 139–170.

———. 1976. The foraging specializations of individual bumblebees. Ecol. Monogr. 46: 105–128.

———. 1981. The energetics of pollination. Ann. Missouri Bot. Gard. 68: 370–378.

———. 1983. Insect foraging energetics. Pp. 187–214 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

——— & P. H. RAVEN. 1972. Energetics and pollination ecology. Science 176: 597–602.

Heithaus, E. R. 1974. The role of plant–animal interactions in determining community structure. Ann. Missouri Bot. Gard. 61: 675–691.

_____, P. A. Opler & H. G. Baker. 1974. Bat activity and pollination of *Bauhinia pauletia:* plant pollinator coevolution. Ecology 55: 412–419.

Hemsley, A. J. & I. K. Ferguson. 1985. Pollen morphology of the genus *Erythrina* (Leguminosae: Papilionoideae) in relation to floral structure and pollinators. Ann. Missouri Bot. Gard. 72: 570–590.

Hernández, H. M. & V. M. Toledo. 1979. The role of nectar robbers and pollinators in the reproduction of *Erythrina leptorhiza*. Ann. Missouri Bot. Gard. 66: 512–520.

_____ & _____. 1982. Floral biology of *Erythrina batolobium* and the evolution of pollination systems in American species of the genus. *Erythrina* symposium IV. Allertonia 3: 77–84.

Heslop-Harrison, J. & Y. Heslop-Harrison. 1983. Pollen–stigma interaction in the Leguminosae: the organisation of the stigma in *Trifolium pratense* L. Ann. Bot. (London) 51: 571–583.

Hesse, M. 1980. Zur stereostruktur der viscin Faden bei Ericaceen und Onagraceen. Beitr. Elektronenmikroskop Direktabb. Oberfl. 13: 211–216.

_____. 1981. Pollenkitt and viscin threads: their role in cementing pollen grains. Grana Palynol. 20: 145–152.

Hobbs, G. A. 1957. Alfalfa and red clover as sources of nectar and pollen for honey, bumble and leaf-cutter bees (Hymenoptera: Apoidea). Canad. Entomol. 89: 230–235.

_____, W. O. Nummi & J. F. Virostek. 1961. Food-gathering behavior of honey, bumble and leaf-cutter bees (Hymenoptera: Apoidea) in Alberta. Canad. Entomol. 93: 409–419.

Hopkins, C. V., A. W. Jevans & R. Boch. 1969. Occurrence of octadeca-trans-2, cis-9, cis-12-trienoic acid in pollen attractive to the honeybee (Hymenoptera, Apoidea). J. Kansas Entomol. Soc. 36: 248–252.

Hopkins, H. C. 1983. The taxonomy, reproductive biology and economic potential of *Parkia* (Leguminosae: Mimosoideae) in Africa and Madagascar. Bot. J. Linn. Soc. 87: 135–167.

_____. 1984. Floral biology and pollination ecology of the neotropical species of *Parkia*. J. Ecol. 72: 1–23.

Hopper, S. D. 1981. Foraging behaviour of Megachilid bees on *Swainsonia canescens* (Fabaceae) and its coevolutionary implications. West Austral. Naturalist 15: 8–11.

Humphries, C. J. & V. A. Funk. 1984. Cladistic methodology. Pp. 323–362 *in* V. H. Heywood & D. M. Moore (editors), Current Concepts in Plant Taxonomy. Academic Press, London & Orlando.

Hutton, E. M. 1960. Flowering and pollination in *Indigofera spicata, Phaseolus lathyroides, Desmodium uncinatum* and some other tropical pasture legumes. Empire J. Exp. Agric. 28: 236–243.

Ignasiak, T. & K. Lesins. 1972. Carotenoids in petals of some perennial *Medicago* sp. Biochem. Syst. 1: 97–100.

Ingham, J. L. 1981. Phytoalexin induction and its taxonomic significance in the Leguminosae (subfamily Papilionoideae). Pp. 599–626 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Inouye, D. W. 1980. The terminology of floral larceny. Ecology 61: 1251–1253.

Irwin, H. S. & R. C. Barneby. 1981. Cassieae. Pp. 97–106 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Ishikura, N. & M. Shibata. 1973. Paper chromatographic survey of anthocyanins in Leguminosae: 2. On the flower pigments of *Mucuna sempervirens*. Bot. Mag. (Tokyo) 86(1001): 1–4.

Jain, K. L. & R. P. Kapil. 1980. Foraging rhythm of megachilid bees in relation to the flowering of *Medicago sativa* L. and *Parkinsonia aculeata* L. Indian Bee J. 42: 35–38.

Jany, E. 1950. The piercing of scarlet runner flowers by bumble bees. Z. Angew. Entomol. 32: 172–183.

Janzen, D. H. 1971. Euglossine bees as long-distance pollinators of tropical plants. Science 171: 203–205.

_____. 1974. Swollen-thorn acacias of Central America. Smithsonian Contr. Bot. 13: 1–

_____. 1981. The defenses of legumes against herbivores. Pp. 951–977 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Johnson, R. A. 1986. Intraspecific resource partitioning in the bumblebees *Bombus ternarius* and *B. pennsylvanicus*. Ecology 67: 133–138.

Jones, C. E. 1978. Pollinator constancy as a pre-pollination isolating mechanism between two sympatric species of *Cercidium*. Evolution 32: 189–198.

_____ & S. L. Buchmann. 1974. Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. Animal Behav. 22: 481–485.

_____ & R. J. Little (editors). 1983. Handbook of Experimental Pollination Biology. Scientific and Academic Editions. New York.

Jones, D. A. 1974. Co-evolution and cyanogenesis. Pp. 213–242 *in* V. H. Heywood (editor), Taxonomy and Ecology. Academic Press, London.

———, S. G. Compton, T. J. Crawford, W. M. Ellis & I. M. Taylor. 1986. Variation in the color of the keel petals in *Lotus corniculatus* L. 3. Pollination, herbivory and seed production. Heredity 57: 101–112.

Joubert, G., J. N. Tasel & A. Delaude. 1977. Pollinisation du trèfle violet diploide (*Trifolium pratense* L.) par les Apoides et particulièrement par les abeilles domestiques hybrides (*Apis mellifica caucasica* × *A. mellifica ligustica*). Apidologie 8: 259–280.

Kambal, A. E., D. A. Bond & G. Toynbee-Clarke. 1976. A study of the pollination mechanism in field beans (*Vicia faba* L.). J. Agric. Sci. (Cambridge) 87: 519–526.

Kamil, A. C. 1978. Systematic foraging by a nectar-feeding bird, the amakihi (*Loxops virens*). J. Comp. & Physiol. Psychol. 92: 388–396.

Kay, Q. O. N. 1978. The role of preferential and assortative pollination in the maintenance of flower colour polymorphisms. *In:* A. J. Richards (editor), The Pollination of Flowers by Insects. Linnean Soc. Symp. No. 6. Academic Press, London.

———. 1987. Ultraviolet patterning and ultraviolet-absorbing pigments in flowers of the Leguminosae. Pp. 317–354 *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

———, H. S. Daoud & C. H. Stirton. 1981. Pigment distribution, light reflection and cell structure in petals. Bot. J. Linn. Soc. 83: 57–84.

Keeler, K. H. 1985. Extrafloral nectaries on plants in communities without ants: Hawaii. Oikos 44: 407–414.

Keighery, G. J. 1982. Bird-pollinated plants in Western Australia. Pp. 77–89 *in* J. A. Armstrong, J. M. Powell & A. J. Richards (editors), Pollination and Evolution. Royal Botanic Gardens, Sydney.

Kenrick, J. & R. B. Knox. 1981a. Post-pollination exudate from stigmas of *Acacia* (Mimosaceae). Ann. Bot. (London) 48: 103–106.

——— & ———. 1981b. Structure and histochemistry of the stigma and style of some Australian species of *Acacia*. Austral. J. Bot. 29: 733–745.

Kevan, P. G. 1972. Floral colors in the High Arctic with reference to insect flower relations and pollination. Canad. J. Bot. 50: 2289–2316.

———. 1978. Floral coloration, its colormetric analysis and significance in anthecology. Pp. 51–78 *in* A. J. Richards (editor), The Pollination of Flowers by Insects. Linn Soc. Symp. No. 6. Academic Press, London.

———. 1983. Floral colors through the insect eye: what they are and what they mean. Pp. 3–30 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

———. 1984. Pollination by animals and angiosperm biosystematics. Pp. 271–292 *in* W. F. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

——— & M. A. Lane. 1985. Flower petal microtexture is a tactile cue for bees. Proc. Natl. Acad. Sci. U.S.A. 82: 4750–4752.

Kinghorn, A. G. & S. J. Smolenski. 1981. Alkaloids of Papilionoideae. Pp. 585–598 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Kirk, W. D. J. 1985. Floral display in *Vicia faba*, and the distribution of a flower thrips, *Kakothrips pisivorus*. Entomol. Exp. Appl. 38: 233–238.

Knox, R. B., R. Marginson, J. Kenrick & A. J. Beattie. 1986. The role of extrafloral nectaries in *Acacia*. Pp. 295–307 *in* B. Juniper & R. Southgate (editors), Insects and the Plant Surface. Arnold, London.

Koeppen, R. & H. H. Iltis. 1962. Revision of *Martiodendron* (Cassieae–Caesalpiniaceae). Brittonia 14: 191–209.

Koptur, S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. Amer. J. Bot. 66: 1016–1020.

———. 1983. Flowering phenology and floral biology of *Inga* (Fabaceae: Mimosoideae). Syst. Bot. 8: 354–368.

———. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevation gradient. Ecology 66: 1639–1650.

Koul, A. K., A. K. Wakhlu & R. Mangotra. 1983. Preliminary observations on the pollination mechanism of some species of *Crotalaria* L. Acta Bot. Indica 11: 188–193.

Kreitner, G. L. & E. L. Sorensen. 1983. Erect glandular trichomes of *Medicago scutellata* (L.) Mill.: gland development and early secretion. Bot. Gaz. (Crawfordsville) 144: 165–174.

Lack, A. J. 1982. The ecology of flowers of chalk grassland and their insect pollinators. J. Ecol. 70: 773–790.

Lackey, J. A. 1979. Sketches of flower dissections in Phaseoleae (Fabaceae, Faboideae). Iselya 1: 19–53.

_____. 1980. Sketches of flower dissections in American Phaseolinae (Leguminosae–Papilionoideae). Iselya 1: 167–186.

_____. 1981. Phaseoleae. Pp. 301–327 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

LAMONT, B. 1979. Extrafloral nectaries in Australian plants, with special reference to *Acacia*. Pp. 15–18 *in* Annual Report 2, Mulga Research Centre, 1978. Western Australian Institute of Technology, Mulga Park.

LANGENHEIM, J. H. 1981. Terpenoids in the Leguminosae. Pp. 627–655 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____, W. H. STUBBLEBINE, D. E. LINCOLN & C. E. FOSTER. 1979. Implications of variation in resin composition among organs, tissues and populations in the tropical legume *Hymenaea*. Biochem. Syst. Ecol. 6: 299–313.

LASSEIGNE, A. 1979. Studies in *Cassia* (Leguminosae-Caesalpinioideae) 3. Anther morphology. Iselya 1: 141–160.

LEPAGE, M. & R. BOCH. 1968. Pollen lipids attractive to honeybees. Lipids 3: 530–534.

LEPPIK, E. E. 1966. Floral evolution and pollination in Leguminosae. Ann. Bot. Fenn. 3: 299–308.

_____. 1977. Floral Evolution in Relation to Pollination Ecology. Today & Tomorrow's Publishers, New Delhi.

LERTZMAN, K. P. & C. L. GASS. 1983. Alternative models of pollen transfer. Pp. 474–489 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

LEVIN, D. A. 1972. Competition for pollinator service: a stimulus for the evolution of autogamy. Evolution 26: 668–669.

_____. 1976. The chemical defences of plants to pathogens and herbivores. Annual Rev. Ecol. Syst. 7: 121–159.

_____. 1978. Pollinator behaviour and the breeding system of plant populations. Pp. 133–150 *in* A. J. Richards (editor), The Pollination of Flowers by Insects. Linn. Soc. Symp. No. 6. Academic Press, London.

_____. 1979. Pollinator foraging behavior: Genetic implications for plants. Pp. 131–153 *in* O. T. Solbrig, S. Jain, G. B. Johnson & P. H. Raven (editors), Topics in Plant Population Biology. Columbia Univ. Press, New York.

_____ & H. W. KERSTER. 1969. The dependence of bee-mediated pollen and gene dispersal upon plant dispersal. Evolution 23: 560–571.

_____ & M. NIEDZLEK. 1971. Pollinator flight directionality and its effect on pollen flow. Evolution 25: 113–118.

LEWIS, G. P. & T. S. ELIAS. 1981. Mimoseae. Pp. 155–168 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

LIENER, I. E. 1979. Phytohemagglutinins. Pp. 567–598 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

LIMA, H. C. DE. 1982. Revisão taxonômica do gênero *Vatairea* Aublet (Leguminosae—Faboideae). Arch. Jard. Bot. Rio de Janeiro 26: 173–214.

LINDAUER, M. 1967. Communication Among Social Bees. Antheneim, New York.

LINSLEY, E. G. 1978. Temporal patterns of flower visitation by solitary bees with particular reference to the southwestern United States. J. Kansas Entomol. Soc. 51: 531–546.

LITTLE, R. J. 1983. A review of floral food deception mimicries with comments on floral mutualism. Pp. 294–309 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

LLOYD, D. G. & C. J. WEBB. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. 1. Dichogamy. New Zealand J. Bot. 24: 135–162.

LOPER, D. G. & A. M. LAPIOLI. 1971. Photoperiodic effects on the emanating of volatiles from alfalfa (*Medicago sativa* L.) florets. Pl. Physiol. (Lancaster) 49: 729–732.

LORD, E. M. & L. U. KOHORN. 1986. Gynoecial development, pollination and the path of pollen tube growth in the tepary bean, *Phaseolus acutifolius*. Amer. J. Bot. 73: 70–78.

LUBIS, S. H. A., S. SASTRAPRADJA, I. LUBIS & D. SASTRAPRADJA. 1977. Genetic variation of *Mucuna pruriens* (L.) DC. 1. Floral biology and pollination mechanism. Ann. Bogor. 6: 133–141.

LUCAS, S. A. & W. L. THEOBOLD. 1982. Observations of flowering behavior in selected species of *Erythrina* in cultivation in Hawaii. Allertonia 3: 85–119.

LUSH, W. M. 1980. Floral morphology of wild and cultivated cowpeas. Econ. Bot. 33: 442–447.

McCann, C. 1952. The Tui and its food plants. Notornis 1952: 6–14.

McCrea, K. D. & M. Levy. 1983. Photographic visualisation of floral colors as perceived by honeybee pollinators. Amer. J. Bot. 70: 369–375.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants, Agricultural Handbook No. 496. Agricultural Research Service. U.S. Department of Agriculture, Washington, D.C.

McKey, D. 1979. The distribution of secondary compounds within plants. Pp. 56–133 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Macior, L. W. 1971. Co-evolution of plants and animals—systematic insights from plant-insect interactions. Taxon 20: 17–28.

———. 1974. Behavioral aspects of coadaptations between flowers and insect pollinators. Ann. Missouri Bot. Gard. 61: 760–769.

Majak, W., R. Neufeld & J. Corner. 1980. Toxicity of *Astragalus miser* v. *serotinus* to the honeybee. J. Apicultural Res. 19: 196–199.

Marginson, R., M. Sedgely & R. B. Knox. 1985. Structure and histochemistry of the extrafloral nectary of *Acacia terminalis* (Salisb.) Macbride (Leguminosae, Mimosoideae). Protoplasma 127: 21–30.

———, ———, T. J. Douglas & R. B. Knox. 1985. Structure and secretion of the extrafloral nectaries of Australian acacias. Israel J. Bot. 34: 91–102.

Meeuse, B. J. D. 1979. The opening and closing of flowers. Univ. Wash. Arbor. Bull. 42: 29–35.

Menichini, F., F. Della Monache & G. B. Marini Bettolo. 1982. Flavonoids and rotenoids from Tephrosieae and related tribes of Leguminosae. Pl. Med. 45: 243–244.

Meudt, W. J. (editor). 1982. Strategies of Plant Reproduction. Allanheld Osmun, London.

Michener, C. 1962. An interesting method of pollen collecting by bees from flowers with tubular anthers. Revista Biol. Trop. 10: 167–175.

Milton, S. J. & E. J. Moll. 1982. Phenology of Australian acacias in the SW Cape, South Africa, and its implications for management. Bot. J. Linn. Soc. 84: 295–327.

Moldenke, A. R. 1976. California pollination ecology and vegetation types. Phytologia 34: 305–361.

———. 1979. Pollination ecology within the Sierra Nevada. Phytologia 42: 223–282.

Moral, R. del. 1979. Mimicry in plants. Univ. Wash. Arbor. Bull. 42: 2–7.

Morse, D. H. 1976. Predatory risk to insects foraging at flowers. Oikos 46: 223–228.

Mosquin, T. 1971. Competition for pollinators as a stimulus for the evolution of flowering times. Oikos 22: 398–402.

Mulligan, G. A. & P. G. Kevan. 1973. Color, brightness and other floral characteristics attracting insects to the blossoms of some Canadian weeds. Canad. J. Bot. 51: 1939–1952.

Murrell, D. C., D. T. Tomes & R. W. Shuel. 1980. Studies of nectar production and floral attractiveness to honeybees of varieties of birdsfoot trefoil. Lotus Newslett. 11: 11.

Nair, P. K. K. & R. B. Tewari. 1975. On the occurrence of the keeler appendage in the flowers of *Phaseolus mungo* L. and *Indigofera enneaphylla* L. New Bot. 2: 91–93.

Narang, A. K. 1977. Some interesting features in the flower of *Crotalaria* and *Tephrosia* species. Curr. Sci. 46: 353–354.

Neff, J. L., B. B. Simpson & A. R. Moldenke. 1977. Resource of utilization systems: flowers–flower visitor system. Pp. 204–224 *in* G. H. Orians & O. T. Solbrig (editors), Convergent Evolution in Warm Deserts. Dowden, Hutchinson & Ross, Stroudsburg.

New, T. R. 1983. Wasps, weevils and wattles: some insect effects on inflorescence and predispersal seed mortality in Australian acacias. Bull. Groupe Int. Étude Mimos. 11: 56–57.

———. 1984. A Biology of Acacias. Oxford Univ. Press, Oxford.

Nielsen, I., T. Baretta-Kuipers & P. Guinet. 1984. The genus *Archidendron* (Leguminosae-Mimosoideae). Opera Bot. 76: 5–120.

Nongonierma, A. 1976. Contribution à l'étude biosystématique du genre *Acacia* Miller en Afrique occidentale: 2. Caractères des inflorescences et des fleurs. Bull. Inst. Fondam. Afrique Noire, Sér. A, Sci. Nat. 38: 487–642.

Numata, M. & H. Tanaka. 1982. Changes in the pollination type composition as related to plant succession. Jap. J. Bot. 32: 207–212.

Nye, W. P. & G. E. Bohart. 1962. Observations of the visits of honey bees and bumble bees to bladder senna *(Colutea arborescens)*. Ann. Entomol. Soc. Amer. 55: 259–260.

O'Dowd, D. J. 1982. Pearl bodies as ant food: an ecological role for some leaf emergences of tropical plants. Biotropica 14: 40–49.

Oster, G. & B. Heinrich. 1976. Why do bumblebees major? A mathematical model. Ecol. Monogr. 46: 129–133.

Ostler, W. K. & K. T. Harper. 1978. Floral ecology in relation to plant species diversity in the Wasatch Mountains of Utah and Idaho. Ecology 59: 848–861.

Pardha Saradhi, P. & H. Y. Mohan Ram. 1981. Some aspects of floral biology of *Cassia fistula* L. (the Indian laburnum): part 1. Curr. Sci. 50: 802–805.

Pazy, B. 1984. Insect induced self-pollination. Pl. Syst. Evol. 144: 315–320.

Pedersen, W. M. 1953. Environmental factors affecting nectar secretion and seed production in alfalfa. Agron. J. 45: 359–361.

_____ & G. E. Bohart. 1953. Factors responsible for the attractiveness of various clones of alfalfa to pollen-collecting bumblebees. Agron. J. 45: 548–551.

_____ & F. E. Todd. 1949. Selection and tripping in alfalfa clones by nectar-collecting honeybees. Agron. J. 41: 247–249.

Pennell, F. W. 1948. Taxonomic significance of an understanding of floral evolution. Brittonia 6: 301–308.

Penny, J. H. J. 1983. Nectar guide color contrast: a possible relationship with pollination strategy. New Phytol. 95: 707–721.

Pereira-Noronha, M. R., I. S. Gottsberger & G. Gottsberger. 1983. Biologia floral de *Stylosanthes* (Fabaceae) no Cerrado de Botucatu, Estado de São Paulo. Revista Brasil. Biol. 42: 595–605.

Periasamy, K. & C. Sampoornam. 1984. Studies on the hypanthial tube, androecium and pollination in *Arachis hypogaea* L. Beitr. Biol. Pflanzen 58: 403–411.

Perry, D. R. & A. Starrett. 1980. The pollination ecology and blooming strategy of a neotropical emergent tree, *Dipteryx panamensis*. Biotropica 12: 307–313.

Peter, J. 1975. Szantofoldi novenyek. Pp. 501–565 *in* L. Halmagyi & B. Keresztesi (editors), A Mehlegelo. Akademiai Kiado, Budapest.

Petkov, V. 1977. Investigation of the nectar-producing qualities of some forage plants. Rasteniev. Nauki 14: 123–133.

Pleasants, J. M. 1983. Structure of plant and pollinator communities. Pp. 375–393 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Plowright, R. C. & L. K. Hartling. 1981. Red clover pollination by bumblebees: a study of the dynamics of a plant–pollinator relationship. J. Appl. Ecol. 18: 639–647.

Polhill, R. M. 1976. Genisteae (Adans.) Benth. and related tribes (Leguminosae). Pp. 143–368 *in* V. H. Heywood (editor), Botanical Systematics, Volume 1. Academic Press, London.

_____. 1981a. Indigofereae. Pp. 289–291 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____. 1981b. Sophoreae. Pp. 213–230 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____ & P. H. Raven (editors). 1981. Advances in Legume Systematics, Volume 1, 2. Royal Botanic Gardens, Kew.

_____ & J. E. Vidal. 1981. Caesalpinieae. Pp. 81–95 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson & A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Rev. Ecol. Syst. 11: 41–65.

Primack, R. B. 1982. Ultraviolet patterns in flowers, or flowers as viewed by insects. Arnoldia 42: 139–146.

Proctor, M. C. F. 1978. Insect pollination syndromes in an evolutionary and ecosystemic context. Pp. 105–116 *in* A. J. Richards (editor), The Pollination of Flowers by Insects. Linn. Soc. Symp. No. 6. Academic Press, London.

_____ & P. F. Yeo. 1973. The Pollination of Flowers. Collins, London.

Pyke, G. H. 1980. The foraging behaviour of Australian honeyeaters: a review and some comparisons with hummingbirds. Austral. J. Ecol. 5: 343–369.

Ramirez, N., C. Sobrevila, N. X. de Endrech & T. Ruíz-Zapata. 1984. Floral biology and breeding system of *Bauhinia benthamiana* Taub. (Leguminosae), a bat-pollinated tree in Venezuelan "Llanos." Amer. J. Bot. 71: 273–280.

Ranta, E. & H. Lundberg. 1980. Resource partitioning in bumblebees: the significance of differences in proboscis length. Oikos 35: 298–302.

———, I. Teras & H. Lundberg. 1981. Phenological spread in flowering of bumblebee-pollinated plants. Ann. Bot. Fenn. 18: 187–200.

Rao, T. A. & S. Das. 1979. Leaf sclereids—occurrence and distribution in the angiosperms. Bot. Not. 132: 319–324.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pp. 305–329 *in* L. Real (editor), Pollination Biology. Academic Press, Orlando.

——— & E. P. Lacey. 1985. Phenological patterns of terrestrial plants. Annual Rev. Ecol. Syst. 16: 179–214.

Real, L. (editor). 1983. Pollination Biology. Academic Press, Orlando.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Pp. 3–54 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Richards, A. J. (editor). 1978. The Pollination of Flowers by Insects. Academic Press, London.

———. 1986. Plant Breeding Systems. George Allen & Unwin, London.

Riedl, R. 1978. Order in Living Systems: A Systems Analysis of Evolution. J. Wiley, Chichester.

Robbertse, P. J. 1974. The genus *Acacia* in South Africa II. With special reference to the morphology of the flower and inflorescence. Phytomorphology 24: 1–15.

Robertson, C. 1929. Phenology of oligolectic bees and favorite flowers. Psyche 36: 112–118.

Rosenthal, G. A. 1986. The chemical defences of higher plants. Sci. Amer. 254: 76–81.

——— & E. A. Bell. 1979. Naturally occurring toxic non-protein amino acids. Pp. 353–385 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

——— & D. H. Janzen (editors). 1979. Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Rotar, P. P., S. J. Park, A. Bromdap & U. Urata. 1967. Crossing and flowering behavior in Spanish clover, *Desmodium sandwicense* E. Mey., and other *Desmodium* species. Hawaii Agric. Exp. Stat. Rep. No. 164.

Ryan, C. A. 1979. Proteinase inhibitors. Pp. 599–618 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Saleh, N. A. M. & M. S. Ishak. 1976. Anthocyanins of some Leguminosae flowers and their effect on colour variation: *Bauhinia variegata, Cassia nodosa* & *Delonix regia*. Phytochemistry 15: 835–836.

Sanduleac, E. V. 1961. *Robinia pseudoacacia*, the most important nectar-yielding tree in Rumania. Arch. Geflugelz. Kleintierk. 10: 219–234.

Sano, Y. 1977. The pollination systems of *Melilotus* species. Oecol. Pl. 12: 383–394.

Sastrapradja, S., S. H. Aminah, I. Lubis & D. S. Sastrapradja. 1975. Studies in the Javanese species of *Canavalia:* 1. Floral biology and cytology. Ann. Bogor. 6: 43–55.

———, S. H. A. Lubis, I. Lubis & D. S. Sastrapradja. 1980. Pollination and pod-setting in winged bean (*Psophocarpus tetragonolobus* (L.) DC.). Ann. Bogor. 7: 89–95.

Saunders, B. J. & W. van Cotthem. 1980. Morphological investigations on the flowers of *Erythrina lysistemon* Hutch. Biol. Jaarb. 48: 126–140.

Schaal, B. A. & W. J. Leverich. 1980. Pollination and banner markings in *Lupinus texensis* (Leguminosae). Southw. Naturalist 25: 280–282.

Schnee, B. K. & D. M. Waller. 1986. Reproductive behavior of *Amphicarpaea bracteata* (Leguminosae), an amphicarpic annual. Amer. J. Bot. 73: 376–386.

Schoener, T. W. 1971. Theory of foraging strategies. Annual Rev. Ecol. Syst. 2: 369–404.

Schrire, B. D. 1984. A taxonomic revision of the tribe *Desmodieae* (Leguminosae-Papilionoideae). M.Sc. thesis. University of Durban-Westville, South Africa.

Scogin, R. 1983. Visible floral pigments and pollinators. Pp. 160–172 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Scott Elliot, G. F. 1891. Notes on the fertilization of South African and Madagascar flowering plants. Ann. Bot. (London) 5: 341–353.

Sell, Y. 1980. Physiological and phylogenetic significance of the direction of flowering in inflorescence complexes. Flora 169: 282–294.

Sherbrooke, W. C. & J. C. Scheerens. 1979. Ant-visited extrafloral (calyx and foliar) nectaries and nectar sugars of *Erythrina flabelliformis* Kearney in Arizona. Ann. Missouri Bot. Gard. 66: 472–481.

Silander, J. A., Jr. 1978. Density-dependent control of reproductive success in *Cassia biflora*. Biotropica 10: 292–296.

Simpson, B. B. & J. L. Neff. 1981. Floral rewards: alternatives to pollen and nectar. Ann. Missouri Bot. Gard. 68: 301–322.

_____ & _____. 1983. Evolution and diversity of floral rewards. Pp. 142–159 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

_____, _____ & A. R. Moldenke. 1977. *Prosopis* flowers as a resource. Pp. 84–107 *in* B. B. Simpson (editor), Mesquite: Its Biology in Two Desert Ecosystems. US/IBP Synthesis Series 4. Dowden, Hutchinson & Ross Inc., Stroudsburg.

Sinha, S. C. 1972. Floral morphology of acacias. Caribbean J. Sci. 11: 137–153.

Small, E., C. W. Crompton, & B. S. Brookes. 1981. The taxonomic value of floral characters in tribe Trigonelleae (Leguminosae) with special reference to *Medicago*. Canad. J. Bot. 59: 1578–1598.

Smolenski, S. J. & A. D. Kinghorn. 1981. Alkaloids of Caesalpinioideae and Mimosoideae. Pp. 579–584 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Solbrig, O. T. & P. D. Cantino. 1975. Reproductive adaptations in *Prosopis* (Leguminosae–Mimosoideae). J. Arnold Arbor. 56: 185–210.

Solereder, H. 1899. Systematische Anatomie der Dicotyledonen. Enke. Stuttgart.

Srivastava, S. C. 1983. Floral biology of Palas: *Butea monosperma* (Lam.) Taub. Indian J. Bot. 6: 57–62.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.

_____. 1970. Adaptive radiation of reproductive characteristics in angiosperms. 1. Pollination mechanisms. Annual Rev. Ecol. Syst. 1: 307–326.

_____. 1973. Evolutionary trends in the inflorescence of angiosperms. Flora 162: 501–528.

_____. 1974. Flowering Plants: Evolution Above the Species Level. Edward Arnold, London & Harvard Univ. Press, Cambridge.

_____. 1984. Mosaic evolution, mosaic selection and angiosperm phylogeny. Bot. J. Linn. Soc. 88: 149–164.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Rev. Ecol. Syst. 12: 253–279.

Stevens, P. F. 1986. Evolutionary classification in botany, 1960–1985. J. Arnold Arbor. 67: 313–339.

Stirton, C. H. 1975. A contribution to knowledge of the genus *Eriosema* (Leguminosae–Lotoideae) in southern Africa (excluding Mozambique and Rhodesia). M.Sc. thesis. University of Natal, South Africa.

_____. 1977. The pollination of *Canavalia virosa* by xylocopid and megachilid bees. Bothalia 12: 225–227.

_____. 1981a. Petal sculpturing in papilionoid legumes. Pp. 771–788 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____. 1981b. The *Eriosema cordatum* complex. II. The *E. cordatum* and *E. nutans* groups. Bothalia 13: 281–306.

Sugden, E. A. 1985. Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records, potential impact on sheep grazing. Great Basin Naturalist 45: 299–312.

Sutherland, O. R. W., G. B. Russell, D. R. Biggs & G. A. Lane. 1980. Insect deterrent activity of phytoalexin isoflavonoids. Biochem. Syst. Ecol. 8: 73–75.

Suthurst, R. W., R. J. Jones & H. J. Schnitzerling. 1982. Tropical legumes of the genus *Stylosanthes* immobilizing and killing cattle ticks. Nature 295: 320–321.

Swain, A. 1979. Tannins and lignins. Pp. 657–682 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Szabo, T. I. & H. G. Najda. 1985. Flowering, nectar secretion and pollen production of some legumes in the Peace River region of Alberta, Canada. J. Apicultural Res. 24: 102–106.

Tanaka, H. 1982. Relationship between ultraviolet and visual spectral guidemarks of 93 species of flowers and the pollinators. J. Jap. Bot. 57: 146–159.

Taubert, P. 1891. *Eminia*, genus novum Papilionacearum. Ber. Deutsch. Bot. Ges. 9: 28–32.

Teuber, L. R., M. C. Albertsen, D. K. Barnes & G. H. Heichel. 1980. Structure of floral nectaries of alfalfa (*Medicago sativa* L.) in relation to nectar production. Amer. J. Bot. 67: 433–439.

Tewari, R. B. & P. K. K. Nair. 1978a. Wing morphology of the flower in *Crotalaria*. Phytomorphology 28: 283–290.

_____ & _____. 1978b. Morphology of spurs in some Papilionaceae. New Bot. 5: 103–108.

_____ & _____. 1979. Wing morphology of the flower in some Galegeae (Papilionaceae). Brenesia 16: 131–138.

THOMSON, J. D. 1981. Skewed flowering distributions and pollinator attraction. Ecology 61: 572–579.
_____. 1983. Component analysis of community-level interactions in pollination systems. Pp. 451–460 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.
THORP, R. W. 1979. Structural, behavioral and physiological adaptations of bees (Apoidea) for collecting pollen. Ann. Missouri Bot. Gard. 66: 788–812.
_____ & J. R. ESTES. 1975. Intrafloral behavior of bees on flowers of *Cassia fasciculata*. J. Kansas Entomol. Soc. 48: 175–184.
TOLEDO, M. V. & H. M. HERNÁNDEZ. 1979. *Erythrina oliviae:* a new case of oriole pollination in Mexico. Ann. Missouri Bot. Gard. 66: 503–511.
TOMS, G. C. 1981. Lectins in Leguminosae. Pp. 561–577 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.
TUCKER, S. C. 1984. Origin of symmetry in flowers. Pp. 351–395 *in* R. A. White & W. C. Dickison (editors), Contemporary Problems in Plant Anatomy. Academic Press, Orlando.
_____, S. R. RUGENSTEIN & K. DERSTINE. 1984. Inflated trichomes in flowers of *Bauhinia* (Leguminosae: Caesalpinioideae). Bot. J. Linn. Soc. 88: 291–301.
TURNER, G. W. 1986. Comparative development of secretory cavities in the tribes Amorpheae and Psoraleeae (Leguminosae: Papilionoideae). Amer. J. Bot. 73: 1178–1192.
TURNER, V. 1983. Non-flying mammal pollination: an opportunity in Australia. Pp. 110–122 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors), Pollination '82. School of Botany, University of Melbourne.
ULUBELEN, A. & T. DOGUC. 1974. Flavonoid compounds from the flowers of *Genista lydia*. Pl. Med. 25: 39–42.
UTECH, F. H. & S. KAWANO. 1975. Spectral polymorphisms in angiosperm flowers determined by differential ultraviolet reflectance. Bot. Mag. (Tokyo) 88: 9–30.
VAN HOVEN, W. 1984. Trees' warning system against browsers. Custos 13: 11–16.
VIDAL, W. N., M. R. R. VIDAL & E. C. DE ALMEIDA. 1983. A polinização de *Cassia laevigata* Willd. Bradea 3: 413–420.
VOGEL, S. 1977. Nektarien und ihre Okologische bedeutung. Apidologie 8: 321–335.
_____. 1978a. Evolutionary shifts from reward to deception in pollen flowers. Pp. 89–96 *in* A. J. Richards (editor), The Pollination of Flowers by Insects. Linn. Soc. Symp. No. 6. Academic Press, London.
_____. 1978b. IV. Floral ecology. Report on the years 1974(73) to 1978. Pp. 453–481 *in* H. Ellenberg, K. Esser, H. Merxmuller, E. Schnepf & H. Ziegler (editors), Progress in Botany/Fortschritte der Botanik 40. Springer-Verlag, Berlin.
VOTH, W. 1982. Die "ausgeborten" Bestaeuber von *Orchis pallens* L. Orchidee (Hamburg) 33: 196–203.
WADDINGTON, C. H. 1975. The Evolution of an Evolutionist. Edinburgh Univ. Press, Edinburgh.
WADDINGTON, K. D. 1979. Divergence in inflorescence height: an evolutionary response to pollinator fidelity. Oecologia 40: 43–50.
_____. 1983a. Floral-visitation sequences by bees: models and experiments. Pp. 461–473 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.
_____. 1983b. Foraging behavior of pollinators. Pp. 213–239 *in* L. Real (editor), Pollination Biology. Academic Press, Orlando.
_____ & HOLDEN, L. R. 1979. Optimal foraging: on flower selection by bees. Amer. Naturalist 114: 179–196.
WADDLE, R. M. & N. R. LERSTEN. 1974. Morphology of discoid floral nectaries in Leguminosae, especially tribe Phaseoleae (Papilionoideae). Phytomorphology 23: 152–161.
WAINWRIGHT, C. M. 1978. The floral biology and pollination ecology of two desert lupines. Bull. Torrey Bot. Club. 105: 24–38.
WASER, N. M. 1983a. Competition for pollination and floral character differences among sympatric species: a review of evidence. Pp. 277–293 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.
_____. 1983b. The adaptive nature of floral traits: ideas and evidence. Pp. 241–285 *in* L. Real (editor), Pollination Biology. Academic Press, Orlando.
WEAVER, N. 1956. Foraging behavior of honeybees on hairy vetch. 1. Foraging method and learning to forage. Insectes Sociaux 3: 537–549.

_____. 1957. The foraging behavior of honeybees on hairy vetch 2. The foraging area and foraging speed. Insectes Sociaux 4: 43–57.

_____. 1965. The foraging behavior of honeybees on hairy vetch 3. Differences in the vetch. Insectes Sociaux 12: 321–326.

_____. 1979. Possible recruitment of foraging honeybees to high-reward areas of some plant species. J. Apicultural Res. 18: 179–183.

_____ & R. M. Weihing. 1960. Pollination of several clovers by honeybees. Agron. J. 52: 183–185.

Webb, C. J. 1984. Constraints on the evolution of plant breeding systems and their relevance to systematics. Pp. 249–270 *in* W. F. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

_____ & D. G. Lloyd. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. New Zealand J. Bot. 24: 163–178.

_____ & J. E. Shand. 1985. Reproductive biology of tree lucerne (*Chamaecytisus palmensis*, Leguminosae). New Zealand J. Bot. 23: 597–606.

Webster, B. D., R. M. Ross & T. Evans. 1982. Nectar and the nectary of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci. 107: 497–503.

Weder, J. K. P. 1981. Protease inhibitors in the Leguminosae. Pp. 533–560 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Wee, Y. C. & A. N. Rao. 1980. Anthesis and variations in floral structure of *Parkia javanica*. Malaysian Forester 43: 493–499.

Wiens, D. 1978. Mimicry in plants. Pp. 365–403 *in* M. K. Hecht, W. C. Steere & B. Wallace (editors), Evolutionary Biology, Volume 11. Plenum Press, New York.

Wijbrandi, J., H. Bruggink, M. G. A. O. Egbrink, J. J. P. O. Loohuis & M. T. Smith. 1984. Nectar robbing of flowers of the broad bean (*Vicia faba* L.) regarded from the view of fitness. Acta Bot. Neerl. 33: 135–136.

Williams, I. H. 1977. Behaviour of insects foraging on pigeon pea (*Cajanus cajan* (L.) Millsp.) in India. Tropical Agric. (Trinidad) 54: 353–363.

Williams, N. H. 1983. Floral fragrances as cues in animal behavior. Pp. 50–72 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York.

Williamson, G. B. 1982. Plant mimicry: evolutionary constraints. Biol. J. Linn. Soc. 18: 49–58.

Wink, M. 1985. Chemical defence of lupines: biological function of quinolizidine alkaloids. Pl. Syst. Evol. 150: 65–81.

Wojciechowska, B. 1972. Pollination and fertilization in *Ornithopis* sp. Genet. Polon. 13: 37–52.

Wyatt, R. 1983. Pollinator–plant interactions and the evolution of breeding systems. Pp. 51–95 *in* L. Real (editor), Pollination Biology. Academic Press, Orlando.

Yeo, P. F. 1972. Floral allurements for pollinating insects. Pp. 51–57 *in* H. F. Van Emden (editor), Insect–Plant Relationships. Blackwell, Oxford.

Zindler-Frank, E. 1987. Calcium oxalate crystals in legumes. Pp. 279–316 *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

Appendix. Framework of Guidelines for a Study of Pollination Biology.

Guide to main sections

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General references on pollination: Baker & Hurd, 1968; Macior, 1971, 1974; Proctor & Yeo, 1973; Leppik, 1977; Neff et al., 1977; Proctor, 1978; Richards, 1978, 1986; Vogel, 1978b; Faegri & van der Pijl, 1979; Armstrong et al., 1982; Jones & Little, 1983; Real, 1983; Waser, 1983b; Barth, 1985.

Selected source references for floral morphology and pollination in the Leguminosae: Dahlgren, 1963; Leppik, 1966; McGregor, 1976; Polhill, 1976; Frankel & Galun, 1977; Lackey, 1979, 1980; Allen & Allen, 1981; Arroyo, 1981; Polhill & Raven, 1981; Small et al., 1981; Adey, 1982; New, 1984.

Selected references of detailed accounts of pollination biology in the Leguminosae: Heithaus et al., 1974; Simpson et al., 1977; Hernández & Toledo, 1979, 1982; Toledo & Hernández, 1979; Cruden & Hermann-Parker, 1979; Perry & Starrett, 1980; Koptur, 1983; Bernhardt et al., 1984; Hopkins, 1984; Webb & Shand, 1985; Hedstrom & Thulin, 1986.

I. POLLINATOR INTERACTIONS AT THE PLANT COMMUNITY LEVEL

General references: Levin, 1972; Heithaus, 1974; Moldenke, 1976; Ranta et al., 1981; Thomson, 1981, 1983; Pleasants, 1983; Rathcke, 1983; Waser, 1983a.

A. Community structure

1. Communities are endowed with coordination, interdependence, and homeostasis. Their structure is defined by certain parameters such as species diversity, dominance patterns, trophic structure, position within community structure (e.g., understory vs. canopy), and diversity of reproductive types.

2. The degree of environmental heterogeneity and any association with the successional status of the community (Moldenke, 1976; Numata & Tanaka, 1982).

3. Altitudinal effects on changes in the type of pollinator visiting a species (e.g., Burquez & Sarukhán, 1980).

B. Population structure in the community context

1. Relative abundance and dispersion of plants in the community (i.e., rarity, distantly spaced individuals or patchiness) in terms of:

a) Response to pollinator foraging behavior.

b) Response to flower herbivores (e.g., are surplus male flowers being produced to avoid loss of fitness as flowers are consumed?). Many factors other than pollination biology, however, might affect abundance and dispersion.

2. Distances between populations and corresponding levels of pollen transfer. (Gene exchange is rare between populations separated by a few hundred meters.)

3. The degree of similarity in the pollination biology between populations of any one species. (This can change with demography, community structure, and population ecology.)

C. Niche dynamics and competitive interactions

1. Niche breadth

a) Niche breadth is the average proportion of resources (in this case pollinators) used by each species in relation to species diversity. For species with similar requirements, niches should differ from one another temporally and/or spatially. Even for minor differences in ecological requirements, there can be selection for maintaining certain genotypes in the population and not allowing the establishment of hybrids between them.

b) Mutualistic interactions; for example, two patchy or widely distributed species using the same pollinator might flower together for display to attract that pollinator.

c) The degree of pollinator constancy to a search-image of a flower and any association with two closely related species flowering shortly after one another (e.g., Ranta et al., 1981)?

2. Equilibria. Richards (1986) noted that for any species of flowering plant, a suite of flower characteristics has evolved which is coadaptive with respect to an equilibrium point of reproductive strategy. This strategy is based for a niche where the species has its own characteristic flowering time, flowering height above ground, means of donating and receiving pollen, means of attracting visitors, etc. Coexisting species in a habitat are likely to have different equilibrium points of reproductive strategy.

3. Perturbations and competitive interactions

a) New competitive interactions may arise from the exclusion or accommodation of species, with a resulting divergence or contraction of niches.

b) Climatic effects, e.g., weather patterns and their effect on flowering periods. Water stress has a strong selective effect on high nectar reward flowers, influencing flowering phenology and habitat preference.

4. Interspecific competition for pollinators and changes in niche breadth.

a) Interference competition: where pollinator movements between individuals of two species reduces the effectiveness of pollinator visits (i.e., competition through interspecific pollen flow).

b) Exploitation competition: where pollinator visits to one species reduce the number of visits to another. The effect of introduced and naturalized plants on competition for pollinators.

5. Niche overlap and mechanisms for avoiding competition by niche displacement.

a) Avoidance of interference competition:

(1) by reducing the amount of temporal overlap of competing species and by the degree of spatial isolation between them. (There is a tendency for pollinators to move to nearest neighbors of the inflorescences they have just left.)

(2) by the promotion of floral constancy through differences in floral morphology, foraging heights in the vegetation, or by traplining (Janzen, 1971).

b) Avoidance of exploitation competition where pollinators and time of flowering are shared:

(1) by the type of pollinator used; for example, competition through pollinator preference. (Floral diversity in relation to the diversity of available pollinators.)

(2) by the time of flowering; that is, temporal flowering divergence (time niches) and sequential blooming through competition (Mosquin, 1971).

(3) by an increase in the overall attractiveness to pollinators relative to other flowering species in the community.

(4) by partitioning the time of day during which rewards, floral signals, and their configuration are presented.

(5) Pollen- and nectar-providing species may be avoiding competition by offering different resources to the pollinators.

D. Synchrony of flowering within species

Flowering might be matinal, late afternoon, crepuscular, nocturnal, or all day. The individuals of a species might flower at the same or at different times. (See Davis & Evans, 1980; Bawa, 1983; Koptur, 1983; Bernhardt, 1983).

1. Selection for asynchrony
   a) By competition through limited pollinator availability or pollinator services in species-rich communities. (Selection might be for reduced geitonogamy or selfing.)
   b) By differences in optimal male and female reproductive phases.
2. Selection for synchronous flowering, promoting mass attraction of pollinators and development of their social interactions that facilitate pollen flow (e.g., group foraging, territoriality, or the attraction of predators to large pollinator guilds [Gentry, 1978]).

E. Flowering phenology of a species in the context of overall community phenology (e.g., Linsley, 1978; Agren & Fagerstrom, 1980)

1. Annual variation (i.e., year-to-year variation in the flowering pattern of a species).
2. Seasonal variation, e.g., flowering before or during the leaf phase. (In dry environments it may be beneficial to partition the energy drain of photosynthesis and that of flower and nectar production [Feinsinger et al., 1979]).
3. Phenology of the pollinators and their relative abundance over space and time (i.e., seasonal synchronicity between plants and their pollinators; see Robertson, 1929).

Changes in pollination biology of a species might occur in response to changes in pollinator diversity; that is, reduced diversity and increased mixing of pollen loads might lead to an increase in rewards offered by plants (Feinsinger et al., 1982).

F. Reproductive success in a community context

1. Pollination success, and the ratio of total number of flowers produced to those that are pollinated.
2. Reproductive success and the ratio of total number of flowers produced to those developing into fruit (e.g., Stephenson, 1981; Bawa & Webb, 1984; Hopkins, 1984).
3. Economic abortion of flowers and sexual selection for the optimization of male and female reproductive success. For example, excess functionally male flowers may be produced (for added attraction, greater reproductive success, or because of herbivore pressure); or abortion may occur to reduce the expense of producing fruit or to thin out inflorescences and reduce the mechanical restraints of fruit development (see Hopkins, 1984).
4. The flowering period in relation to fruiting time in terms of reproductive effort.
5. The effect of the percentage loss of floral resources from herbivores and opportunists on reproductive success (e.g., through nectar and pollen robbery or theft).

II. PLANT POPULATION STRUCTURE AND GENE FLOW (relative abundance and dispersal of plants)

General references: Levin & Kerster, 1969; Levin, 1978; Silander, 1978; Evenson, 1983; Handel, 1983; Richards, 1986; Sackville Hamilton, this volume.

A. Size, structure, and shape of population

1. Plant population size; that is, the overall number of genets (Richards, 1986).
2. The proportion of ramets to genets in the population (Richards, 1986).
3. Shape of the population and its influence on the rate of dilution of incoming pollen.
4. Edge effect; that is, the influence on pollen flow by pollinator foraging along the edges rather than in the center of the population.
5. The proportion of self-incompatibility to self-compatibility in relation to population size, density, and shape; that is, in natural populations there are complex patterns of different outcrossing rates.
6. Population structure and differences in sex ratio in plants.

B. Population density

1. Flower density in relation to pollinator flight dynamics and foraging behavior (Levin & Niedzlek, 1971).
2. Increased flower density and any association with:
   a) a decrease in the amount of nectar rewards per flower, thus enhancing pollinator movement between plants.
   b) copious nectar flow and attraction of large numbers of pollinators.
3. Flower density and levels of geitonogamy, particularly in clones with many ramets.
4. Effect of flower density on the overall breeding system.
   a) High flower density and the persistence of self-incompatibility.
   b) Local pollen dispersal patterns due to restricted gene flow (thus enhancing genetic drift).
   c) Low density populations and genotypically controlled self-fertility.
   d) "Large" plants in clones (many ramets) and any association with greater levels of selfing.
5. Change in plant density and spread from year to year and the consequences for population size.
6. Population density and herbivore or pathogen pressures.
7. Population density and its effect on seed dispersal strategies.

C. Spatial distribution and position of plants in a population

1. The distribution and size of plants in the immediate vicinity of the individual; that is, are populations diffuse or clumped?
2. Spatial pattern and any evidence of high or low density preferring pollinators.
3. Position effects; that is, the rate of dilution of incoming pollen between outer and inner individuals in a large population.
4. Clonal growth; that is, flowers becoming increasingly surrounded by inflorescences of the same plant.
5. Environmental heterogeneity and its influence on pollen flow in the population.

D. Pollinator dynamics and genetic neighborhood

1. The degree of nearest-neighbor pollinator movement and its influence on the formation, persistence, and spread of clumps.
2. Population neighborhoods; that is, areas of random gene exchange (panmixis) within a population. Most populations are not panmictic units.

E. Reproductive success in a population context

1. *K*- and *r*-type reproductive efficiency strategies (Gadgil & Solbrig, 1972) or facultative switching between them in plants growing in fluctuating environments (e.g., in those subject to repeated fires; Schrire, 1984).
2. Population density and its effect on subsequent components of reproductive success; that is, the proportion of sexual to vegetative reproductive allocation and the amount of seed set.
3. Pollen–ovule ratios and reproductive effort.

III. FORAGING PATCH STRUCTURE (Levin, 1978)

A. Patch structure

1. This is defined as a spatially discrete natural assemblage of suitable host plants, of one to a few species, presenting a similar syndrome to pollinators.
2. Patches are pollinator defined; that is, they are parts of the environment recognized by a particular pollinator for foraging.
3. Patch structure is variously interpreted by different species and types of pollinators.
4. There is a relatively low likelihood of interspecific pollen flow with a relatively large patch size composed of high-quality plants, persistent for a number of years. Interspecific pollen flow is most likely when the patch size is relatively small and composed of unpredictable resources.

B. Patch dynamics and pollinator behavior

1. Influence of the temporal and spatial dynamics of patch size on pollinator choice, and the relative magnitude of differences in rewards between patches (e.g., Weaver, 1979).
2. Choice switching—that is, once foraging becomes unprofitable due to declining resources or due to an increase in pollinator density.
3. A decreased diversity in reward structure between patches and its association with lowered pollinator constancy or specialization to those patches.
4. Declining patch size and any association with lowered floral constancy and pollinator specificity.
5. The influence of successional status on patch size. During colonization the patch size is small; it peaks as the species becomes established, and tails off as the community matures.
6. Patch size is correlated with longevity of the species; colonizers may have a very large patch size but may be short-lived.
7. Density regulation of the patch is initially by abiotic factors and interspecific competition, and later by herbivores and pathogens.
8. The interplay of pollinator specialization, as an evolutionary adaptation, and constancy, as an ecological adaptation, and levels of interspecific cross pollination.
9. High specificity and constancy, with only a small proportion of pollinator flights occurring between patches, is usually associated with a low possibility of interspecific pollen flow and hybridization. If this is general on both accounts, then the possibility is higher.
10. Periodic and nonuniform disturbance and any association with a greater probability of interspecies pollen flow.

C. Choice, number, and sequence of patches visited

1. The choice, number, and sequence of patches visited in a foraging bout is influenced by the abundance of resources in the community.
2. The proportion of pollinator flights between congeneric species in different patches is a crude indicator of a likely level of interspecific cross pollination.

IV. POLLINATOR ENERGETICS AND FORAGING BEHAVIOR

General references: Schoener, 1971; Heinrich & Raven, 1972; Heinrich, 1975, 1976, 1981, 1983; Frankie et al., 1976; Levin, 1979; Waddington, 1979, 1983a, b; Pyke, 1980; Frankie & Haber, 1983; Carpenter, 1983; Richards, 1986.

Selected references for detailed studies in the Leguminosae: Weaver, 1956, 1957, 1965, 1979; Hobbs et al., 1961; Frankie & Baker, 1974; Heithaus et al., 1974; Williams, 1977; Cruden & Hermann-Parker, 1979; Feinsinger et al., 1979; Jain & Kapil, 1980; Hopper, 1981; Beresford & Marginson, 1983; Hopkins, 1984; Hedstrom & Thulin, 1986.

Further references are cited in the text as examples.

A. Diet preferences of pollinators

1. Pollinators with generalist or specialist food preferences. This should not only be assessed by the kinds of flowers visited but also by the number and sequence of different flower visits relative to flower availability.
2. Nectar robbers and any effect on legitimate pollinator behavior because of ready access to the nectar, made possible through a hole in the floral parts.

B. Direction, distance, and duration of flight to foraging patch

1. A patch area is a spatially discrete, suitable host plant or assemblage of plants that may be comprised of one to a few species at any one time.
2. Distance of the hive or nest from the foraging patch.
3. Number of foraging patches visited per trip.
4. Pulsed visitation by a pollinator to a foraging patch, and any association with an optimal time for nectar replenishment. Is the pollinator returning before competing groups locate the resource (Heithaus et al., 1974)?

C. Behavior of pollinators within the overall foraging patch

1. Number of flower species visited.
2. Method of floral choice; that is, by scouting or by previous experience, by "majoring" and "minoring" (Oster & Heinrich, 1976; Heinrich, 1976, 1983), or by example followed from others (e.g., trail marking in meliponine bees [Lindauer, 1967; Esch, 1967]). The behavior towards a particular plant species depends on the rewards of other plants in the surrounding community at that time.
3. The sequence of visits may be influenced by the spatial and temporal distribution of flower species.
4. Degree of floral constancy (Baker & Hurd, 1968; Jones, 1978; Bernhardt et al., 1984); that is, the fidelity of individual pollinators to site, species, visiting sequence, or height in the vegetation (Heithaus et al., 1974; Waddington, 1979; Hopkins, 1984). In *Senna pallida* (Frankie & Coville,

1979), the most common pollen-collecting visitors were *Centris* and two *Xylocopa* spp. (solitary bees). *Centris* preferred high-level foraging in the plant despite rewards being artificially increased below. *Xylocopa* species switched to the newly increased rewards.

5. Flight patterns between plants; e.g., characterized by long flights or movement between very near neighbors.

6. The presence of behavior patterns such as traplining (Heithaus et al., 1974; Feinsinger et al., 1979), group foraging (Frankie & Baker, 1974; Heithaus et al., 1974; Feinsinger et al., 1979; Hopkins, 1984), territoriality, and aggression (e.g., Frankie & Baker, 1974; Gill, 1978; Jones, 1978), pollinator response to predators (Gentry, 1978), or competition; and any association of these with the displacement of individuals to other food sources on neighboring conspecifics, promoting outcrossing.

D. Behavior of pollinators within individual plants

1. Number of inflorescences visited per plant.

2. Direction of flights between inflorescences; that is, flight distances and changes in angle of interflower flights. The combination indicates the relative efficiency of foraging returns.

3. Selective foraging; that is, preference shown for particular inflorescences or flowers (e.g., avoidance of flowers that have lost attractiveness through color changes [Schaal & Leverich, 1980], or preferences shown for particular corolla depth [Ranta & Lundberg, 1980]).

4. Approach and landing behavior (e.g., Jones & Buchmann, 1974; Jones, 1978; Toledo & Hernández, 1979; Hopkins, 1984); for example, hovering or perching and associated orientation to inflorescences and/or flowers.

5. Ability to manipulate flowers to obtain rewards, and any evidence of the importance of optimal size and/or skill (Wainwright, 1978).

6. Time spent at a flower.

7. Behavior in or on the flower, for example, milking or vibration of anthers (Buchmann, 1983).

8. Proportion of time spent at an inflorescence; that is, the search time between flowers (Hopper, 1981).

9. Proportion of flowers visited per inflorescence.

10. Direction and pattern of successive movement within an inflorescence, as influenced by flower orientation, sequential receptivity of flowers, and change in floral reward gradient along the inflorescence (e.g., Hopper, 1981; Haddock & Chaplin, 1982; Lloyd & Webb, 1986).

11. Evidence of avoidance of flowers already visited.

V. THE MOST EFFECTIVE POLLINATOR (Foraging behavior as it influences pollen flow and the enhancement of foraging returns and energetics)

A. Generalist or specialist pollinators

1. Foraging on generalist or specialist flowers.

2. Pollinators may be generalists as species, but as individuals they specialize. Many pollinators might be sharing the same foraging area, containing a variety of plants in flower, but individuals of one or more species might be utilizing the flowers of different plant species in different proportions while foraging at a particular site (Heinrich, 1983).

B. Quality, quantity, and distance of pollen carryover

1. Amount of pollen carryover (Lertzman & Gass, 1983); that is, the number of subsequent flowers to which the pollen of one flower is carried (e.g., Plowright & Hartling, 1981).

2. The effect of pollen carryover on real as opposed to observed flight distances; that is, pollen might be carried further than the next plant visited.

3. The purity of pollen loads carried by individual pollinators (e.g., Heithaus et al., 1974; Feinsinger et al., 1979; Bernhardt et al., 1984); that is, relatively pure pollen loads could be collected, not because pollinators are flower constant but because they have site fidelity to an area where there are at that time no other plants in flower (Heinrich, 1983).

4. Degree of geitonogamy (Arroyo, 1976).

C. Efficiency of pollination

1. Frequency of contact with the reproductive organs in the flower.

2. Differential pollen placement on the pollinator in relation to the contact area with the stigma (degree of anther exsertion), and interaction with dichogamy and/or herkogamy (see under breeding systems) in the flower (e.g., Heithaus et al., 1974).

D. Pollinator behavior dependent on the availability, quality, quantity, and efficient use of floral resources

1. Timing of visit in relation to the availability of rewards and/or stigma receptivity.

2. Length of visit in relation to the quantity and/or quality of rewards.

3. Regularity of pollinator visits and the temporal and spatial predictability of resources. Facultative constancy might be induced in pollinators by the availability of high quality, predictable, and well-defended rewards.

4. Efficiency at satisfying metabolic (nectar) requirements while foraging for pollen. Pollinator movement is a balance between energetic costs and rewards for foraging.

5. Ability to switch resources in an ever-changing resource base; that is, to detect intraspecific differences in flowering phenology, and nectar flow volume and quality. This is associated with the amount of interplant movement and foraging energetics (e.g., Frankie & Haber, 1983).

6. Skill at locating the most rewarding flowers; that is, being flexible in selecting plant species and having the ability to switch resources and optimize the forming of a search-image of a flower.

7. Skill at learning to handle flowers efficiently to harvest rewards, for example, in difficult flowers that limit access to rewards. Selection for specific pollinator skills or adaptations (e.g., pollinator trophic apparatus and access to nectaries [Koul et al., 1983]).

8. Pollinator size and energetics, and the nutritional content of and accessibility to the reward; that is, hidden rewards have a higher energy content, requiring higher energy-demanding pollinators to harvest them.

9. The energetics of foraging modes (hovering or perching) and the number of flowers visited or the quantity of rewards obtained per unit time.

E. Competitive ability

1. Competitive ability with other effective pollinators and its relative importance in terms of these.

2. Suboptimal foraging; that is, pollinators might be visiting flowers even though they offer no rewards (e.g., mimics).

VI. FLORAL VISITORS (Pollination vectors and other visitors)

Selected references for detailed descriptions of floral visitors to legumes: Frankie & Baker, 1974; Heithaus et al., 1974; Green & Bohart, 1975; Thorp & Estes, 1975; Frankie et al., 1976; Simpson et al., 1977; Cruden & Hermann-Parker, 1979; Feinsinger et al., 1979; Hernández & Toledo, 1979; Toledo & Hernández, 1979; Perry & Starrett, 1980; Lack, 1982; Bond & Poulsen, 1983; Koptur, 1983; Turner, 1983; Bernhardt et al., 1984; Hopkins, 1984; Sugden, 1985.

A. Pollination vectors (biotic or abiotic)

1. Agent (wind) or animal vector.
2. Type and sex of pollinator.
3. Sex differences and differences in foraging behavior and choice of food plant.
4. Solitary or social, associated with particular behavior patterns (e.g., group behavior).
5. Polylectic, oligolectic, or monolectic food preferences or foraging patterns.
6. Degree of division of labor in pollinator type (e.g., in advanced social insects, foragers have a greater opportunity to develop foraging specializations and efficiency).
7. Nest materials and clues to the reward preference of the pollinator.
8. Demography of pollinator species relative to plant species.
9. Availability of the pollinator in relation to the presentation of rewards by a flowering plant species.

B. Other floral visitors

1. Opportunists
2. Nectar robbers: use force by biting a hole through the floral parts (Inouye, 1980). (Primary robbers make holes, and secondary robbers make use of existing ones.)

a) *Phaseolus coccineus* (Jany, 1950), *Vicia atropurpurea,* and *V. faba* (Bohart, 1960). *Bombus* spp. were responsible for piercing of flowers and robbing nectar, while other bees were noted to forage from the holes.

b) *Colutea arborescens* (Nye & Bohart, 1962). Nectar robbing occurs by honeybees that cannot trip the large flowers.

c) *Sophora microphylla* (Clinch et al., 1972). Bees often collect nectar through holes made in the corolla by birds.

d) *Vicia* (Macior, 1974). Fertility was unaffected by nectar-foraging through corolla perforations.

e) *Trifolium pratense* (Joubert et al., 1977). *Apis* workers robbed flowers of nectar through holes pierced at the base of corolla tubes by *Terrestribombus*.

f) *Erythrina poeppigiana* (Feinsinger et al., 1979). Many flowers were pierced and nectar removed. Hummingbirds may have been secondary robbers, since bees might have made the holes.

g) *Erythrina leptorrhiza* (Hernández & Toledo, 1979). *Diglossa baritula,* the Mexican flower piercer, robbed nectar by perforating the calyx. Also two species of hummingbirds pierced calyces and robbed nectar in a different manner.

h) *Erythrina oliviae* (Toledo & Hernández, 1979). Hummingbirds were noted at the flowers, perching on the stamens while piercing the corolla to rob nectar.

i) *Vicia faba* (Wijbrandi et al., 1984). Short-tongued *Bombus* spp. were unable to reach the intrastaminal nectaries, and holes were bitten in the calyx and corolla, in the upper part of the flower at a level where the nectaries could be reached.

j) *Chamaecytisus palmensis* (Webb & Shand, 1985). Honeybees robbed flowers of nectar

through holes cut into the calyx by *Bombus terrestris*. This does not generally damage the ovary.

k) *Andira surinamensis* (*Thurn s.n.*, K.), *A. parvifolia* (*Regnell 3.465*, K), *Vatairea lundellii* (*Matuda s.n.*, MO), *Ormosia flava* (*Ducke 1714*, K), *Alexa cowanii* (*Stergios et al. 3347*, PORT), *Diplotropis martiusii* (*Fróes 22971*, NY), and *Clathrotropis macrocarpa* (*Ducke 1033*, K). These herbarium specimens of sophoroid legumes were noted for having many calyces with holes bitten through them at the level of the hypanthium (Stirton, pers. comm.).

3. Nectar thieves. No force is used; the thief uses legitimate pollinator openings or other means of access, but a mismatch of morphology precludes pollination.

a) *Cajanus cajan* (Williams, 1977). Wasps took nectar from flowers without touching the anthers or stigma.

b) *Bauhinia ungulata, Hymenaea courbaril,* and *Inga vera* (Haber et al., 1981). Various ant species visited the flowers for nectar. (This may be another plant–ant interrelationship that is an aspect of defense against herbivores.)

c) *Parkia* (Hopkins, 1983, 1984). Information is given on nectar and pollen thieves.

4. Pollen robbers that damage floral tissues when obtaining pollen. *Vicia faba* (Kirk, 1985; Bond & Poulsen, 1983). Damage, including pollen predation, occurs by flower thrips, reducing seed set and possibly exerting a selective force on floral life-span and display. (Thrips may be associated with a possible premature breakdown of the stigmatic cuticle, decreasing the chances of outcrossing, or can be pollinators themselves [Stirton, pers. comm.]).

5. Herbivores.

6. Parasites: ovipositing Hymenoptera or bruchids. Sometimes what appear to be nectar robbers might be insects biting holes in the calyx in order to oviposit in the young ovary (G. P. Lewis, pers. comm.). In *Acacia baileyana* and *A. longifolia* (New, 1983), univoltine wasps lay eggs in the inflorescence buds, axillary buds, or apical foliage. The larvae produce substantial galls, which either prevent inflorescence development, cause abortion, or result in few-flowered inflorescences. These wasps are being used for the biological control of invasive *Acacia* in South Africa (Stirton, pers. comm.).

VII. BREEDING SYSTEMS (Controlling the genetic structure of populations and the patterns of evolution)

General references: Bawa, 1980; Arroyo, 1981; Bawa & Beach, 1981; Grant, 1981; Meudt, 1982; Wyatt, 1983; Webb, 1984; Lloyd & Webb, 1986; Webb & Lloyd, 1986; Richards, 1986.

A. Self-pollination versus cross-pollination

1. Autogamy—pollen donation to the same flower.
2. Allogamy—pollen donation to different flowers.
3. Geitonogamy
   a. Pollen donation in the same ramet (selfing).
   b. Pollen donation in different ramets in the same genet (selfing).
4. Xenogamy—pollen donation between different genets (crossing).

B. Major breeding system types

An increase in pollen/ovule ratios occurs from 1 to 4 below (Cruden, 1977). The major differences between outcrossing species and selfing derivatives are summarized in Wyatt (1983).

1. Obligate autogamy: flowers are cleistogamous, the perianth never opens, and self-fertilization is certain. Cleistogamous flowers may occur in allogamous species growing in uncertain environments, where chasmogamous flowers may fail to set seed (e.g., where subterranean cleistogamous, aerial cleistogamous, and aerial chasmogamous flowers occur together [Schnee & Waller, 1986]).
2. Facultative autogamy: flowers open (chasmogamous), but selfing occurs before or during flower opening and exposure of the stigma to pollinators (Hardin et al., 1972; Wojciechowska, 1972). Self-pollination may need to be induced by pollinator visitation (Sastrapradja et al., 1980; Pazy, 1984).
3. Facultative xenogamy: flowers are regularly self-compatible, protogynous, or homogamous; although some require a pollinator, most self in the absence of or in addition to outcrossing.
4. Obligate xenogamy: flowers are outcrossing, protandrous, or self-incompatible, and most require a pollinator.
5. "Sweepstakes": a gambling, lottery-type breeding system where pollinia or polyads of pollen, having a close fit with a corresponding stigma, give large reproductive returns when pollination is successful (a case of xenogamy with a low pollen/ovule ratio).
6. Facultative switching between xenogamous and autogamous systems in fluctuating environments (for example, in those environments influenced by fire), when variable (outbred) and invariable (inbred) offspring have selective advantages at different times in k- and r-type reproductive strategies (Schrire, 1984).
7. Apomixis and the promotion of genet size.

C. Self-fertilization versus cross-fertilization

Successful fertilization does not necessarily result from successful pollination.

1. Self-incompatibility: plants are polymorphic for the presence of self-incompatibility alleles; pollination involving pollen and stigmas sharing the same self-incompatibility alleles (including self-pollination) results in unsuccessful fertilization.
2. Self-compatibility: plants are monomorphic and without the presence of self-incompatibility alleles; all pollination including self-pollination can result in fertilization. Selection for self-compatibility may not always be causally related to the regulation of genetic uniformity; that is, when genetic uniformity or reduction in gene flow are of selective advantage. It may also increase the probability of successful fertilization, be of advantage in the face of pollen unpredictability or due to lack of pollinators, lower the cost of reproduction by producing fewer flowers, or promote higher levels of seed production (Bawa, 1974).

D. Sexuality and/or apomixis (vegetative reproduction and agamospermy)

E. Degree of temporal or spatial separation between pollen donation (from anthers) and pollen reception (on stigmas) reducing the levels of autogamy

1. Separation in time—dichogamy (Lloyd & Webb, 1986). Single or multiple floral units may be involved; that is, intrafloral or interfloral (in the cases of monoecy or dioecy, respectively).
   a) Complete separation
      (1) Protandry: pollen is removed from the anthers before the stigmas attain receptivity.
      (2) Protogyny: the stigmas become receptive before the anthers release their pollen.
   (Homogamy occurs if the male and female phases function at the same time.)
   b) Incomplete separation
      (1) Degree of separation; that is, the average interval between pollen presentation and stigma receptivity.

(2) Degree of synchrony in flowers of one species (asynchronous, hemisynchronous, synchronous; multi- or one cyclic).

2. Separation in space—herkogamy (Webb & Lloyd, 1986).

a) Homomorphic: all flowers are of one form, hermaphroditic.

(1) Unordered: the pollinator usually has many contacts with the pollen and stigma within a flower, but in no particular sequence.

(2) Ordered: the pollinator usually has a single, ordered contact with the pollen and stigma.

(a) Approach: the stigmas are placed forward, in the pollinators' path.

(b) Reverse: the pollen is placed forward, in the pollinators' path.

(c) Movement: the stigma and/or pollen is moved into or out of presentation position by the pollinator (e.g., Heithaus et al., 1974).

b) Reciprocal: flowers are of two or more forms, all hermaphroditic, but differing in presentation position of pollen and/or stigmas; that is, heteromorphy (distyly with pin and thrum conditions [Ganders, 1979; Richards, 1986]) or enantiostyly (Buchmann, 1983).

c) Interfloral: flowers are of two or more forms, the pollen and stigmas are presented partly or wholly in separate flowers (monoecy or dioecy).

F. Level of geitonogamy

This is dependent on the patterns of pollen movement between flowers, the size of the genets (number of ramets and area of ground covered), and the number of donating and receiving flowers open in each ramet.

G. Sexual systems

1. Hermaphroditism: plants bear only bisexual flowers. (Flowers may be functionally unisexual or dimorphic [Ramirez et al., 1984].)

2. Monoecism: separate male or female flowers occur on a hermaphrodite plant; most flowers tend to be dichogamous and/or herkogamous. Some plants may be able to change their proportion of sex expression to optimize fitness.

a) Andromonoecism: hermaphrodite and male flowers occur in the same genet; this may be advantageous if seed set is restricted by inadequate pollination. There may be an association with andromonoecy and specialization for pollinators that are very large relative to stigma size (Heithaus et al., 1974).

b) Gynomonoecism: hermaphrodite and female flowers occur in the same genet; this may be advantageous if fruit size or seed number is restricted by male expenditure.

3. Dioecism: the genet usually expresses only one sex, only male or female, both within the flower and the plant (Bawa, 1980).

a) Androdioecism: the coexistence of separate hermaphrodite and male genets.

b) Gynodioecism: the coexistence of separate hermaphrodite and female genets.

4. Unstable genotypic expression and the occurrence of:

a) Subgynoecy: where wholly female genets coexist with gynomonoecious genets.

b) Subandroecy: where wholly male genets coexist with andromonoecious genets.

c) Polygamy: where genets with male only, female only, hermaphrodite only, and gynomonoecious and/or andromonoecious flowers coexist.

d) Degree of separation of function of flowers both within and between inflorescences on the same individual.

e) Level of floral abnormality in a species and a possible role in reproductive success (Bose et al., 1983).

VIII. PHENOLOGY OF FLOWERING (timing, duration, and frequency of flowering at individual and population levels)

General references: Gentry, 1974; Frankie et al., 1974; Moldenke, 1979; Bawa, 1983; Rathcke & Lacey, 1985; Richards, 1986.

Selected references for detailed studies in the Leguminosae: Simpson et al., 1977; Feinsinger et al., 1979; Milton & Moll, 1982; Augspurger, 1983; Borchert, 1983; Koptur, 1983; Hopkins, 1984.

A. Patterns of flowering (Gentry, 1974)

1. Mass patterns: 1) big bang, 2) cornucopia, 3) multiple bang.
2. Extended patterns: 1) steady state, 2) modified steady state.

Mass and episodic flowering, or extended flowering periods, and their association with pollinator fidelity and foraging strategies.

B. Seasonality of flowering

C. Number and duration of flowering cycles

Supra-annual, annual, or episodic. The latter may be advantageous when either weather conditions or pollination opportunities are uncertain.

D. Positional phenology within an individual plant

Phenology of flowers and inflorescences relative to the entire individual.

E. Flowering time and duration differences between sexes

1. In dioecious species
2. Between male, female, or hermaphrodite phases in individuals
3. Changes in time of the proportion of hermaphrodite to male or female flowers within individuals.

F. Direction of flowering in an inflorescence (Sell, 1980; Hopkins, 1984)

G. Persistence of flowering

1. Single flowers (Srivastava, 1983)
2. Inflorescences
   a) Average lifespan
   b) Number of flowers opening daily

H. Anthesis and the time of maturity of the sexual organs

1. Time of anthesis.
2. Time of anther dehiscence (Chow, 1974; Heithaus et al., 1974; Sastrapradja et al., 1975; Wainwright, 1978; Koul et al., 1983).
3. Time of stigma receptivity.
4. Period of pollen viability (Periasamy & Sampoornam, 1984).

I. Time and duration of reward presentation

1. Time of pollen availability.
2. Time and rate of nectar flow (Pedersen, 1953).
3. Time needed to replenish resources (i.e., are rewards continuous or intermittent?).

J. Influences of time, duration, and frequency on flowering phenology (Bawa, 1983)

1. Time
   a) Competition for pollinators among species
   b) Selection against interspecific gene flow
   c) Availability of pollinators
   d) Nature of rewards
   e) Selection for the optimization of life history traits other than flowering (e.g., resource allocation to periods when there is no vegetative activity, to when there is less chance of seed herbivores, or to when seed dispersal agents are available).
2. Duration
   a) Flower herbivores
   b) Level of geitonogamy and outcrossing
   c) Control over relative investment in flowers and fruits
   d) Predictability of pollinators
   e) Competition for pollinators
   f) Selection against interspecific gene flow
   g) Opportunities for colonization
   h) Position in the canopy
3. Frequency
   a) Seed predation
   b) Predictability of the environment
   c) Temporal pattern of resource availability

IX. PLANT MECHANISMS FOR PROMOTING THE TRANSFER OF THE MALE GAMETOPHYTE TO THE FEMALE GAMETOPHYTE

A. Tripping mechanisms in papilionoid flowers (see under deterrent and defensive features).

B. Androecium (see Owens & Stirton, Kenrick & Knox, Owens, and Shivanna & Owens, this volume).

1. Stamen number: by increasing the number of stamens (e.g., in the Mimosoideae and in the genus *Swartzia*, Papilionoideae).

2. Polymorphy

a) Heteranthery or functionally different stamens (Vogel, 1978a) and the provision of separate feeding and reproductive pollen; the presence of cryptic polliniferous anthers as a possible response to increasing pollination efficiency or to use different vectors.

(1) *Bauhinia pauletia* (Heithaus et al., 1974). Only five of the ten stamens produce pollen. The remaining five atrophy but look like productive anthers. Anthers are loosely attached, becoming easily detached by pollinators or wind.

(2) *Swartzia* sp. (Vogel, 1978a) have spatially separated, voluminous feeding androecia with small anthers poor in pollen.

(3) *Parkia javanica* (Wee & Rao, 1980). A range of stamen types occur, from staminodes to filaments with vestigial anthers and no pollen, to normal anthers.

b) Alternately long and short filaments: their differential growth, different times of anther dehiscence and/or anther dimorphy; and their relation to the efficiency of pollen transfer (e.g., Granby, 1980; Koul et al., 1983).

(1) *Chamaecrista fasciculata* (Hardin et al., 1972). Anther dimorphy occurs, with eight anterior and two posterior anthers of different size and shape. The latter have much greater amounts of pollen, which is all viable, while the smaller anthers provide feeding pollen to bees.

(2) *Cassia silvestris* (Fontenelle, 1979). Two morphological subtypes of anthers were noted which do not occur in the same flower. Differences in and the nonsynchronous development of pollen in the sterile and fertile anthers was also noted in each flower.

(3) *Arachis hypogaea* (Periasamy & Sampoornam, 1984). A hypanthium tube, consisting of the fusion of calyx, corolla, and androecium, elongates rapidly with the style as the ovules ripen. Four stamens with elongate anthers and short filaments (in the open flower) alternate with and are topped by four stamens with globose anthers and longer filaments. Two staminodes are also usually present. In the bud the anthers are in the reverse position, the elongate anthers dehiscing first, followed by the globose anthers. The filaments of the latter then elongate (nonsimultaneously), pushing through the space bounded by the elongate anthers and lifting the pollen mass to the level of the stigma.

3. Stamen fusion and the degree of accuracy of pollen transfer to the vector. (The vexillary stamen may have a possible independent functional role.)

4. Presentation: the degree of stamen exsertion, and expansion, thickening, or curvature of the filaments in relation to the pollination mechanism.

5. Anther modifications

a) Specialized adaptations regarding anthers; for example, hygroscopic pollen cups (Koeppen & Iltis, 1962).

b) Anther connective development and a possible role in pollination; for example, forming a synandrium together with coherent anthers (Irwin & Barneby, 1981).

c) Position of anther attachment to the filaments (fixed or versatile) and the direction of dehiscence (e.g., introrse, lateral, etc.).

d) Anther appendages (glands [e.g., Simpson et al., 1977] or tufts of hairs) assisting in pollen transfer to the vector; hairs and a role in the separation of pollen from self-compatible stigmas.

e) Specialized anther dehiscence; that is, explosive or poricidal (above or below), and requiring pollinator vibration or milking (Michener, 1962) to obtain the pollen (Delgado & Sánchez, 1977; Fontenelle, 1979; Lasseigne, 1979; Buchmann, 1983; Vidal et al., 1983).

6. Pollen state

a) Whether pollen is sticky or dry in relation to the means of transfer to the vector, that is, dry and forming a pollen cloud in explosively tripping flowers or in wind pollinated plants (Janzen, this volume), or sticky as in bird pollinated plants (Hemsley & Ferguson, 1985).

There may be an association between pollen state and the often long periods between anther dehiscence and anthesis.

b) Provision of pollen with viscin threads (Cruden & Jensen, 1979) or ropy pollenkitt (Hesse, 1980, 1981; Cruden & Hermann-Parker, 1979), where stigma to pollen contact is unlikely due to small stigma size and large vector surface.

(1) *Jacqueshuberia* (Graham in Arroyo, 1981): presence of true viscin threads in pollen.

(2) *Erythrina* (Hemsley & Ferguson, 1985): pollen from tubular hummingbird-pollinated flowers is dustlike and dry, while that from gaping passerine bird-pollinated flowers is sticky and difficult to separate because of pollenkitt on the surface.

7. Exine surface of pollen and any association with pollenkitt or with pollinator type (Ferguson & Skvarla, 1982; Hemsley & Ferguson, 1985). *Harpalyce, Camoensia, Millettia,* and *Dahlstedtia* (Ferguson & Skvarla, 1982) are genera of trees and shrubs with large red or white flowers adapted for pollination by birds or bats and having coarsely rugulate or verrucate pollen. Insect-pollinated genera in such primitive tribes, however, have simple reticulate or perforate surface sculpturing. In more recent tribes, for example in *Lessertia* and *Sutherlandia,* such a correlation does not hold.

8. Size of pollen dispersal unit (monads or polyads) and the efficiency of reproduction (Bernhardt et al., 1984).

9. Electrostatic attraction: between pollen and the vector (Erickson & Buchmann, 1983).

10. Pollen ripening: dark keel petal tips and any association between increased solar absorption and pollen maturation (Crawford & Jones, 1986).

C. Gynoecium (see Owens & Stirton, Kenrick & Knox, Owens, and Shivanna & Owens, this volume)

1. Stigma

a) Type: wet, dry, papillate, or nonpapillate (Kenrick & Knox, 1981b; Heslop-Harrison & Heslop-Harrison, 1983).

b) Shape, size, and position: bilobed, peltate, capitate, minute, and hardly distinguishable from the style, tubular, porate, ciliolate-crateriform, or funnel-shaped; position, terminal, introrse, or peripheral.

c) Presence of a stigmatic cuticle: preventing self-pollination until ruptured by flower tripping.

(1) *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1983).

(2) *Phaseolus acutifolius* and *Vicia faba* (Lord & Kohorn, 1986). Various stigmatic cuticles in legumes are discussed, as is cuticle fragmentation and selfing in the cleistogamous flowers of the above species.

d) Stigmatic exudates and capture, rehydration, and nutrition of pollen grains (Baker et al., 1973; Kenrick & Knox, 1981a). *Acacia retinodes* (Bernhardt et al., 1984): postpollination stigmatic exudate occurs very briefly.

2. Style

a) Length and curvature in relation to pollen reception by the stigma (Dwyer & Chen, 1971; Kambal et al., 1976); enantiostyly (Fontenelle, 1979; Buchmann, 1983).

b) Shape and form: constricted, geniculate, swollen, grooved, or with entases, either physically restricting the growth of some pollen tubes or aiding in the transfer of pollen to vectors during pollination.

c) Style elongation as a physical isolating mechanism allowing only the most vigorous pollen tubes to reach the ovary (e.g., Periasamy & Sampoornam, 1984).

d) Conducting tissue: solid or hollow (lysigenously or shizogenously formed; Owens, this volume), stylar exudates and the promotion of pollen tube selection and growth.

e) Vestiture: presence and pattern of various hairs on the style (barbate) or around the stigma (penicillate), and the presence of well-developed stigmatic papillae.

(1) Separating dehisced pollen from self-compatible stigmas (e.g., Webb & Shand, 1985) in untripped flowers.

(2) Assisting in the transfer of pollen to the vector (Hedstrom & Thulin, 1986).

(3) Assisting in the transfer of pollen from the vector to the stigma.

(a) *Mucuna pruriens* (Lubis et al., 1977). Although the stigma is moist one day prior to anthesis, it is only considered fully receptive when the papillae are produced (at the time of simultaneous anther dehiscence and anthesis).

(b) *Lupinus arizonicus* and *L. sparsiflorus* (Wainwright, 1978). A ring of papillate cells around the stigma keeps the pollen from reaching the stigmatic surface. Pollen has to be forced onto the stigma to get a response for germination.

(c) *Psophocarpus tetragonolobus* and *Mucuna pruriens* (Sastrapradja et al., 1980). Hairs around the stigma keep away self pollen. The stigma has stigmatic papillae in addition to hairs, and insect tripping is necessary for selfing to occur.

3. Mechanical isolation of the stigma and self pollen due to the distal shape of the keel petals which form a canal for pollen transfer during tripping. In *Vicia faba*, Kambal et al. (1976) and Bond & Poulsen (1983) described a mechanism to prevent selfing in spite of early anther dehiscence. The keel petal ridge (canal), the stylar hairs, and the stigmatic papillae that only rupture and produce exudate after tripping isolate self pollen from the stigma. Differences in the shape of the distal end of the keel occur in auto-fertile and nonselfing lines, allowing the passage of self pollen to the stigma in the former but not in the latter.

4. Stipe: differential growth of the stipe may occur during the life of the flower to raise the style and stigma above the other floral parts, thereby enhancing intraspecific pollen flow.

5. Pollen placement: position of the anthers and stigma relative to each other and any selective placement of pollen on the vector where it is most likely to come into contact with the stigma. In *Trifolium pratense* (Plowright & Hartling, 1981), pollen deposition is restricted to the ventral surface of the bumblebee head, where it cannot be groomed off.

X. PLANT FEATURES ATTRACTIVE TO POLLINATORS

Guidance and recognition cues: In the taxon under study, the question should be asked, are any of the points below significant as being long- or short-distance guidance and recognition cues for pollinators?

A. Inflorescences

1. Number: single, few to many; the average number of flowers per inflorescence. In large mass-flowering trees, many flowers are produced only for attraction (Solbrig & Cantino, 1975).

a) *Prosopis chilensis* and *P. flexuosa* (Simpson et al., 1977). A mature individual of either species is estimated to produce ±10,000 inflorescences a year, with about 50–90 flowers opening fresh in each on any one day.

b) *Erythrina oliviae* (Toledo & Hernández, 1979). Trees were very noticeable from long distances when in flower (also flowering occurs when the trees are leafless; one tree was estimated to have a total of 350 inflorescences.

2. Position on the plant: cauliflorous, axillary, terminal or leaf-opposed. Orientation: vertical, horizontal, pendant, flagelliflorous. Positional change with time, affecting attractiveness; height borne in the plant; degree of conspicuousness, that is, obscured by leaves or held away from the leafy parts.

a) *Parkia javanica* (Wee & Rao, 1980). Inflorescences are of rounded heads of flowers at the ends of long hanging peduncles.

b) *Erythrina breviflora* (Arroyo, 1981), *E. poeppigiana* (Ferguson, 1984), *E. crista-galli* (Lucus & Theobold, 1982). The inflorescence branches have a horizontal orientation in the first two species but are pendant with resupinate flowers in the third. All three are adapted to perching bird pollination.

c) *Baptisia leucophaea* and *B. leucantha* (Haddock & Chaplin, 1982). These are two sympatric species. The former flowers earlier, the inflorescences are smaller and are produced as shorter racemes that lie horizontally on the ground. The latter flowers at a time when the grass canopy is closed over, and it produces erect inflorescences.

d) *Erythrina batolobium* and *E. breviflora* (Hernández & Toledo, 1982). The flowers of the former are grouped into vertical inflorescences, are oriented outwards, and are hummingbird-pollinated, while those of the latter are borne in horizontal inflorescences, face inwards toward the primary axis, and are pollinated by perching birds.

e) *Parkia* (Hopkins, 1984). The unit of attraction in bat-pollinated species of *Parkia* is a ball-like capitulum, borne on woody leafless peduncles arising from unbranched or sparsely branched axes. The peduncles may be pendant or erect, and a good diagram is given of the positions of inflorescences in the canopy. Bat-pollinated inflorescences tend to be exposed beyond the foliage. Insect-pollinated species have capitula borne at all angles on short stiff peduncles, and capitula may be included inside the canopy.

f) *Neptunia oleracea* (G. P. Lewis, pers. comm.). A positional change in the inflorescences occurs with time. At first they are erect, but as soon as the lower sterile florets produce attractive yellow staminodes, the inflorescence bends over at a point of articulation on the peduncle. The attractive staminodes are opened out above the fertile florets and a gradation occurs through male flowers to fully hermaphrodite flowers at the apex (which is lowermost after articulation of the inflorescence).

3. Structure, shape, and size: architecture, degree of separation of function of flowers, degree of aggregation of flowers and showiness.

a) *Bauhinia pauletia* (Heithaus et al., 1974). The bat-pollinated flowers are borne on a woody peduncle and are of two types, hermaphrodite and functionally staminate. In the latter the degenerate pistils drop from the flowers at anthesis. The proportion of each flower type varies within individuals from night to night.

b) *Acacia pycnantha* (Ford & Forde, 1976). The structure of the inflorescences is discussed, and pollination occurs by attraction of birds to the nearby extrafloral nectaries.

c) *Melilotus* (Sano, 1977). The inflorescence is the attractive unit.

d) *Erythrina pallida, E. fusca, E. poeppigiana* (Feinsinger et al., 1979). The first species is relatively uncommon in the community and produces small numbers of less conspicuous (showy) flowers. The other two are much more common and produce inflorescences with many showy flowers. This is related to two different pollinator behavior patterns.

e) Mimosoideae and various others (Arroyo, 1981). Large showy inflorescences occur in many legumes where the individual flowers are relatively insignificant (aggregation for greater effect); for example, *Calliandra, Affonsea, Brownea, Strongylodon,* or *Trifolium.* This culminates in genera such as *Dichrostachys, Parkia,* and *Neptunia,* where the heads are separated into fertile and sterile portions, the latter usually with bright attractive staminodes and markedly contrasting in color with the fertile part (e.g., *Dichrostachys cinerea*).

f) *Cassia fistula* (Pardha Saradhi & Mohan Ram, 1981). Attraction occurs by massed inflorescences.

g) *Erythrina* (Lucus & Theobold, 1982). A series of photographs of *Erythrina* inflorescences is given.

h) *Parkia* (Hopkins, 1984). Three basic types of flowers occur in the inflorescences: a staminodal fringe of functionally neuter flowers nearest the peduncle (usually attractive and occasionally used as footholds by pollinators), specialized nectariferous flowers similar to fertile ones but of larger diameter which open less fully, and fertile florets. The details of the

inflorescences and floral characters for 12 species are given. Capitula may be functionally all hermaphrodite, all male, or mixed.

4. Supplementary attraction-only inflorescences. *Acacia karroo, A. nilotica, A. robusta,* and *A. davyi* (Gordon-Gray & Ward, 1975). Plants produce capitula of normal bisexual florets but also a ring of robust florets in the involucel. These open before the capitate inflorescences, are sterile, and have stout staminodes without apical anthers or glands. They are also paler in color and larger than the capitular florets. The capitula are hermaphrodite and male, the former distally placed and grading downward to the latter. The involucral florets are visually attractive and also produce attractive odors. (They may be associated with establishing visiting patterns before the inflorescences open or to attract egg-laying insects.)

5. Color: patterns and contrasts; the attractive enhancement of inflorescences by retaining spent flowers.

a) *Prosopis* (Simpson et al., 1977). In some species the inflorescences are a dull green color but they contrast against the leaves; in others they are bright yellow.

b) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). Spent flowers remain on the inflorescence, enhancing the overall attractive effect.

c) *Parkia javanica* (Wee & Rao, 1980). Distinct color zones occur in the mature inflorescences associated with the sequence of opening of neuter, bisexual, and male flowers (in that order).

d) *Acacia* (Bernhardt, 1983; Bernhardt et al., 1984). The majority of SE Australian species have inflorescences of varying shades of yellow.

e) *Lupinus argenteus* (Gori, 1983). Flowers that lose their individual attractiveness through postpollination color changes are retained on the inflorescence for up to four or five days before wilting, enhancing the overall attractiveness of the inflorescence.

f) *Inga* (Koptur, 1983). Inflorescences with numerous white stamens are the main visual attractant.

g) *Parkia* (Hopkins, 1984). The capitulae may be pink-brown, red, or yellow. They are visually attractive in the late afternoon. In bat-pollinated species there is a correlation between red- or dark-colored capitulae being held out far from the foliage. This enhances the silhouette effect. The paler yellow ones, which are held nearer the foliage, are more conspicuous against the dark foliage. Open capitulae are highly reflective and conspicuous, reflecting moonlight or starlight under natural conditions.

6. Sequential flower maturation: development of flowers along the inflorescences, enhancing its attraction over time.

a) *Lupinus arizonicus* and *L. sparsiflorus* (Wainwright, 1978). Flowers develop spirally along the inflorescence and may approach distinct whorls.

b) *Acacia retinodes* (Bernhardt et al., 1984). Flowers open acropetally in the inflorescences.

c) *Parkia* (Hopkins, 1984). Capitular inflorescences usually open sequentially from the base of each inflorescence branch.

B. Accessory organs: leaves, bracts (bracteoles), or extrafloral nectaries enhancing the attractiveness of the inflorescence to pollinators.

1. *Vicia atropurpurea* (Bohart, 1960). Extrafloral nectaries occur at the base of the stipules, close to the axillary flowers. Pollen attractiveness is low, and the floral nectar is often robbed through the calyx by bumblebees.

2. *Liparia* (Bos, 1967). The bracts are petaloid, light green to light yellow, large, and turning conspicuously red or maroon later. (The bracts may be chiefly protective.)

3. Various (Bhattacharyya & Maheshwari, 1971). Extrafloral nectaries occur in the Vicieae,

Phaseoleae, Galegeae, Genisteae, Trifolieae, Hedysareae, Dalbergieae, and Loteae but are absent in the Podalyrieae, Sophoreae, and Swartzieae.

4. *Amherstia* (García, 1975). Showy red bracteoles enhance the attractiveness of the flowers.

5. *Chamaecrista fasciculata* (Thorp & Estes, 1975). Extrafloral nectaries occur in this species, which only offers pollen as a reward. (This may also be an ant–plant association.)

6. *Acacia pycnantha* (Ford & Forde, 1976). Extrafloral nectaries are situated at the base of the petioles and secrete nectar during the flowering period. Only those close to inflorescences attract birds. Insects visiting the nectaries may be an added attraction to birds.

7. *Acacia aneura* (Lamont, 1979). Extrafloral nectaries occur on the phyllodes or leaves, and nectar secretion is closely associated with flowering. Although ants visit the nectaries, the nectar appears to be a pollinator attractant.

8. *Talbotiella, Saraca, Hymenostegia, Maniltoa, Lysidice, Brownea* (Cowan & Polhill, 1981a, b). The bracteoles are showy and they enhance the attractiveness of the flowers. In the latter species, the bracts are also attractive.

9. *Acacia terminalis* (Beresford & Marginson, 1983), *A. pycnantha* (Marginson et al., 1985), and *A. myrtifolia* (Knox et al., 1986). Large extrafloral nectaries are present on the petioles of the leaves but not the phyllodes, producing nectar that ranges in color among individuals from deep crimson to orange, yellow, or green. They are located near the inflorescence and secrete only during the flowering season.

10. *Archidendron* (Nielsen et al., 1984). One section of the genus has flowers subtended by bracts and a ring-shaped extrafloral nectary.

11. Various (Keeler, 1985). A list of legumes with extrafloral nectaries is given. The nectaries are discussed together with the constituents of the nectar.

12. *Pseudeminia comosa* (Stirton, pers. comm.). Conspicuous pink to mauvish brown bracts and bracteoles enhance the attractiveness of the inflorescence.

C. Flowers

1. Size: as an attractive unit on its own (although this may be enhanced through aggregation in an inflorescence); closeness of fit with the size and shape of the pollinator.

a) *Chamaecrista fasciculata* (Hardin et al., 1972). A single showy flower is the attractive unit and is borne on short racemes in the leafy axils. Only one flower per raceme is open at a time (anthesis occurs for only one day).

b) *Genista anglica* (Adey, 1982). The importance of flower size in attracting certain pollinators was noted when medium-sized bees foraged with difficulty on the large flowers. The bees overhung the flowers, pulled them down with their weight, and flew off after only a few visits. Small bees also foraged at the flowers using illegitimate methods, for example, *Lasioglossum* spp. on *Cytisus scoparius*. This also indicates the effectiveness of the tripping mechanism as an isolating feature.

2. Shape: overall shape and recognition by the pollinator; corolla structure and length in proportion to pollinator accessibility and nectary position; the relative importance of the degree of dissection of the flower as opposed to flower outline, and the distinguishing of shapes by insect mosaic vision.

a) *Eriosema* (Stirton, 1975). The process of anthesis is described with reflexion of the standard opening up a path to the nectary. As the standard becomes fully reflexed, this forces the wings to turn from the vertical into the horizontal plane. They are then available as a landing platform for the pollinator.

b) *Melilotus alba* and *M. officinalis* (Johnson, 1986). Two species of *Bombus* were noted foraging, where flower species with short corollas (the above two species) occurred with species having long corollas (*Centaurea maculosa* and *Agastache foeniculum*). The plants

occurred in single and in mixed species stands. In the latter, foragers on species with shorter corollas were found to have shorter proboscises than conspecific foragers on species with long corollas. However, lengths of the proboscis of foragers on the species with a long corolla in single species stands did not differ from those on the species with a short corolla in single species stands. Whenever a choice existed, therefore, the bees selected species having a corolla length most approximating their proboscis length.

3. Position: flowers facing outward or inward relative to the plant axis; inclination relative to the inflorescence axis; resupination; geanthy (flowers held close to the ground).

a) *Baptisia leucophaea* and *B. leucantha* (Haddock & Chaplin, 1982). On the horizontal inflorescence of the early flowering *B. leucophaea*, flowers are parallel to the axis opening towards the apex of the raceme. The earliness of the season meant that only *Bombus* queens were foraging, which are large and relatively awkward. Bees walked between the flowers (less energetically expensive), moving towards the base of the inflorescence and carrying pollen from staminate to pistillate flowers. The plant is self-compatible. *Baptisia leucantha* flowered later in the season, when the worker caste of the bumblebees were active. In the erect inflorescence the flowers open outwards, perpendicular to the peduncle. A differential nectar gradient ensured that pollinators moved up from pistillate to staminate flowers, thus encouraging outcrossing.

b) *Erythrina* (Hernández & Toledo, 1982). The landing perch, which is the resupinate standard petal in gaping, inwardly facing, passerine bird-pollinated flowers, does not occur in the tubular, outwardly facing hummingbird-pollinated flowers.

c) *Bossiaea dentata, Brachysema praemorsum* and other species, *Clianthus, Kennedia,* and *Leptosema* (Keighery, 1982). Australian bird-pollinated flowers differ from the typical bird pollination syndrome (Faegri & van der Pijl, 1979) by having brush or cleistopetalous flowers which are commonly yellow or white (although red often occurs). They possess odors and are borne at ground level (geanthus).

4. Enantiomorphy: left- and right-handed deflection of parts, for example in *Cassia* (s.l.) and *Copaifera* (Arroyo, 1981). This produces a zygomorphic effect based on opposite pairs of flowers on the inflorescence axis; that is, attraction may be based on the effect of two and not individual flowers.

5. Floral mimesis: deceit or mutualistic (Wiens, 1978; Moral, 1979; Williamson, 1982; Little, 1983; Dafni, 1984, 1986).

a) *Acacia retinodes* (Bernhardt, 1983; Bernhardt et al., 1984) presents an example of deceit. The floral reward is commonly absent during some period of the flower's lifespan and occurs when the flower is most receptive to pollination. The period is brief, and the number of flowers bearing rewards always exceeds those that do not. Pollen, the only major reward available, is absent during the female phase in this protogynous species.

b) Bright anther contrasts through the female phase and attraction by deceit.

c) *Lupinus bicolor* (Little, 1983) presents an example of mutualistic mimicry. There is a striking similarity in floral morphology between *Collinsia* (Scrophulariaceae) and papilionoid legumes; that is, *C. parviflora* and *L. bicolor* might be analagous to Mullerian mimics.

(1) Batesian mimicry occurs where a rare nonrewarding species mimics the flowers of a more abundant provider (Pennel, 1948; Yeo, 1972).

(2) Mullerian mimicry occurs where a number of similar looking plants have evolved a common "advertising style" to their mutual benefit (e.g., *Lathyrus vernus* and *Orchis pallens* [Orchidaceae], Voth, 1982; and *Dioclea lasiocarpa* with *Monnina* spp. [Polygalaceae], Ducke, 1901).

6. "Rendezvous attraction" (Richards, 1986); for floral visitors other than pollinators.

Selected references, not quoted elsewhere, where flower structure is discussed in relation to

pollination are: Scott Elliot, 1891; Rotar et al., 1967; Sinha, 1972; Chow & Crowder, 1974; Nongonierma, 1976; Narang, 1977; Couderc & Gorenflot, 1978; Saunders & van Cotthem, 1980.

D. Individual floral parts: size, shape, number, position, and color as specific attractants.

1. Calyx or individual sepals

a) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). Some sepals enlarge, becoming reddish, adding to the attractiveness of the flower.

b) *Leonardoxa, Saraca, Bathiaea,* and *Apaloxylon* (Cowan & Polhill, 1981a). Species in these genera have enlarged, showy sepals.

c) *Duparquetia* (Irwin & Barneby, 1981). Two large exterior sepals are aligned on the vertical axis and lie behind the vexillar petal and two interior petaloid sepals.

d) *Crotalaria* (Koul et al., 1983). Modification of the calyx plays a supportive role for heavy insect pollinators. The calyx lobes subtending the keel act as a support for pollination of *C. madurensis* by xylocopid bees.

e) *Dipteryx* and *Pterodon* (G. P. Lewis, pers. comm.). The flower buds in these two genera are protected by two bracts. As the buds expand and the bracts drop off, the two upper calyx lobes expand and add to the attractiveness of the flower, often providing a marked color contrast with the corolla. They are covered with resin glands, which may provide an additional protective role.

2. Corolla or individual petals: besides the obvious attractive role, they may have other functions.

a) *Medicago, Trigonella, Indigofera,* and *Phaseolus* (Tewari & Nair, 1978b). Cylindrical or truncated spurs occur at the proximal ends of the wings or keel and are either hollow at the base, solid, or with specialized secretory tissue at the apex. Insects were found that probed at the secretions. The spurs are either part of the interlocking structure between the wing and keel petals or are part of the attraction system guiding and supporting the insect visitors.

b) *Erythrina oliviae* (Toledo & Hernández, 1979). The very reduced keel and wings form a raised well for the nectar to accumulate.

c) *Macrolobium* (Cowan & Polhill, 1981b). Flowers have a single large petal with 1–4 minute petalodes. The reduction of petals to petalodes is also a feature in the Swartzieae (Cowan, 1981).

d) *Duparquetia* (Irwin & Barneby, 1981). The petals are elaborated, being fimbriate-glandular.

3. Androecium

a) Staminodes: modified attractive stamens, or conspicuous tufts of hairs or knobs (like pollen) on the filaments or connective. In *Parkia javanica* (Wee & Rao, 1980), the staminodes are spongy with a glandular surface and secretory tips (producing a sticky secretion).

b) Heteranthery: the attraction of massed feeding anthers in heterantherous flowers.

(1) *Swartzia* and *Aldina* (Vogel, 1978a). These have a voluminous androecium with small anthers poor in pollen. Trends are noted to partial or total replacement of true anthers and pollination by deceit in the latter genus.

(2) *Senna didymobotrya* and *S. auriculata* (Dulberger, 1981). Heteranthery acts as a guide to the positioning of pollinators on the flowers. The three uppermost stamens have sterile anthers. The rest produce fertile anthers of three types that differ in orientation, size, shape, and pollen production. The anthers are poricidal, requiring the specialized behavior of stamen vibration by the pollinator, *Xylocopa pubescens*. In the first species the two large lateral anthers have a pollinating role, producing pollen picked up on the side of the insect by the curved style. The other anthers, a top group of five and a lower

one, supply pollen to be combed off by the bee and used as brood food. Only the feeding anthers are exposed on the first day the flower opens, although ultimately the pollinating anthers may also supply much of the food.

c) Filaments: color patterns and color contrasts with the corolla; stamens persisting and remaining attractive after the male phase; a possible attractive role of the vexillary stamen in diadelphous androecia, being visible while the others remain hidden in the keel.

(1) Mimosoideae (Arroyo, 1981). Little intrafloral differentiation occurs in the subfamily: conspicuous nectar guides are lacking, corollas tend to be reduced, colors are more uniform, and the stamens are the main visual attractant. Color contrasts are established between the stamens and the corolla, for example in *Calliandra*, where white filaments contrast against red corollas. The stamen filaments may also be bicolored (red and white) in *Calliandra*, associated with pollination by birds during the day and moths at night (G. P. Lewis, pers. comm.).

(2) *Bauhinia ungulata* (Ramirez et al., 1984). Flowers are normally pollinated by bats at night, but as the filaments persist for 2–3 days after pollination, turning from green to red, it is possible that they might attract hummingbirds, which could be effective pollinators in the early evening.

(3) *Amphicarpaea bracteata* (Schnee & Waller, 1986). A change in filament color with time occurs in both fertilized and unfertilized flowers, which are pollinated by many insects and hummingbirds by day and by bats and moths at night.

d) Anthers: attractive anthers or color contrasts with the corolla or filaments.

(1) *Chamaecrista fasciculata* (Hardin et al., 1972). The long, curved, deep purple anthers contrast against the very short yellowish filaments.

(2) *Prosopis* (Simpson et al., 1977). The bright yellow pollen contrasts with the dull green flowers and appears bright to insects.

(3) *Dimorphandra* group (Polhill & Vidal, 1981). The sagittate (horseshoe-shaped) anthers are the most attractive part of the flower.

(4) *Stryphnodendron rotundifolium* (G. P. Lewis, pers. comm.). Flowers have white filaments, blood red anthers, and white anther glands.

4. Gynoecium: by direct attraction of the stigma/style or by stigmatic exudate.

a) *Ceratonia siliqua* (Stirton, pers. comm.). The stigma/style is a visual cue in dioecious species such as above.

b) *Acacia* (Kenrick & Knox, 1981a, b). The possible production of a postpollination stigmatic exudate and the attraction of pollinators to the subsequent male phase of flowering. Pollination results in the massive secretion of an aqueous stigmatic exudate which persists for some time. This was noted in nine species where anther glands are absent.

c) *Parkia* (Hopkins, 1984). The specialized nectariferous flowers have extruded styles which possibly function to help coalesce the droplets of copious nectar.

5. Hypanthium/disc: the possibility of fleshy discs (i.e., in *Dialium* or *Copaifera*) contributing to attraction. In *Acrocarpus* (Polhill & Vidal, 1981), the greenish petals contrast with the red hypanthium.

E. Flower movement

1. Nastic: flowers opening and closing in response to particular presentation times.

2. Positional: changes in flowers or floral parts over time (not postpollination movements); for example, stamen elongation, growth of the stipe, style elongation or differential anther dehiscence, and any association with the partitioning of floral functions.

a) *Eriosema* (Stirton, 1975). In the African species, the flowers usually hang downwards before they open, rise to the horizontal plane as they open, and return to their former position when they close.

b) *Vatairea* (Lima, 1982). The pollination process is discussed, an important part of which is the process of anthesis. Consecutive movements of the petals, that is, vexillum reflexion and movement of the wings through 90° from the vertical to the horizontal plane (providing a landing platform for pollinators), are associated with the flowers becoming attractive only at a certain time of the day.

3. Whole flower movement: for example, in the wind; and the attraction to insect mosaic vision through the flicker effect (Walt, 1937, in Adey, 1982).

F. Flower color

General references: Macior, 1971; Jones & Buchmann, 1974; Kevan, 1972, 1978, 1983; Primack, 1982; McCrea & Levy, 1983; Scogin, 1983; Barth, 1985; Richards, 1986; Kay, 1987.

1. Overall color: attraction and as a signal for rewards; the presence or absence of color polymorphism. The ontogeny of color is important, since the pigments and/or structure of the epidermal or subepidermal layers might be different for similar colors (Kay et al., 1981; Scogin, 1983).

a) *Medicago* (Ignasiak & Lesins, 1972). Discussion of carotenoid pigments in the petals.

b) *Mucuna sempervirens* (Ishikura & Shibata, 1973). Survey of anthocyanin pigments in the flowers.

c) *Medicago lupulina, Vicia cracca, Melilotus alba, M. officinalis,* and *Lotus corniculatus* (Mulligan & Kevan, 1973). Flower color (including UV), inflorescence structure, and odor production are discussed in terms of their relative importance as attractants. A list of insect visitors is given.

d) *Bauhinia variegata, Cassia javanica* var. *indochinensis,* and *Delonix regia* (Saleh & Ishak, 1976). Discussion of anthocyanins in flowers and their effect on color variation.

e) *Mucuna pruriens* (Lubis et al., 1977). Different forms of the species either have violet or greenish white flowers.

f) *Acacia bidwillii* (Hawkeswood, 1985). Butterflies are attracted to the yellow flowers or moisture in the flowers. They are not obtaining nectar but do effectively carry pollen. Although they are regular visitors, they probably play a minor role in cross pollination.

2. Color combinations: distinct patterns as orientation cues. Color polymorphism in the population and a possible role in preferential and assortative pollination (Kay, 1978).

a) *Eriosema* (Stirton, 1975). Flower color varies from pure yellow, red and yellow, pale pink with purple stripes, red, yellow and orange, to orange and yellow. Characteristic of nearly all taxa is the presence of a semicircular patch of intense yellow situated just below the appendages. Each species in southern Africa has a distinct pattern.

b) *Cajanus cajan* (Williams, 1977). Three distinct color patterns are noted in this species.

c) *Crotalaria* (Tewari & Nair, 1978a). The costae in the petals are sometimes of a different color and are seen as stripes radiating out across the lamina.

d) *Erythrina oliviae* (Toledo & Hernández, 1979). The attractive red color of the inner petals contrasts with the orange of the standard and possibly operates as a nectar guide to birds (which is unusual in ornithophilous flowers).

e) Genistinae (Adey, 1982). Different color patterns are noted in 22 species.

f) *Lotus corniculatus* (Jones et al., 1986). The dark tip of the keel petals is associated with attraction and guidance, for example, in dull overcast conditions as opposed to bright sunlight.

g) Mirbelieae and Phaseoleae subtribe Kennediinae (Stirton, pers. comm.). Genera in these Australian groups contain examples of flowers with startling contrasts in color patterns; for example, one species of *Kennedya* has a shiny black-colored standard with a brilliant yellow, central nectar guide.

h) *Lupinus mutabilis* (G. P. Lewis, pers. comm.). This species has distinctive color patterns.

i) Psoraleeae, *Dolichos, Virgilia,* and *Lotus* (Stirton, pers. comm.). The color of the tip of the keel petals may either be on the outside or the inside of the keel tip, or on both surfaces, and may project above or be obscured by the wing petals. This feature probably has a protective role as well.

3. Nectar guides: presence or absence; their role in leading the vector to the focus for the efficient operation of the pollination mechanism; or leading directly or indirectly to the rewards; or possibly imitating pollen where the anthers are hidden (Penny, 1983).

4. The presence and position of UV light absorbance or reflectance patterns on any part of the flower act as nectar guides or break up otherwise uniform color patterns, making them more variable and conspicuous, particularly to insect mosaic vision. Contrasts in UV absorbance and reflectance in the flower and against the visible background enhances the overall range of color patterns visible to the pollinators.

a) *Caesalpinia eriostachys* and *Parkinsonia aculeata* (Jones & Buchmann, 1974). The UV absorptive patterns on the standard and the staminal parts are used for in-flight orientation by bees, and these occur in addition to visual patterns. In both species the UV patterns are absorptive while the other petals are reflective. In *C. eriostachys* the anthers are absorptive and the filaments reflective. In *P. aculeata* the entire stamen is absorptive. The absorptive patterns approximate a vertical band which bisects the flower, thus providing the orientation cue.

b) *Astragalus cibarius* and *A. utahensis* (Green & Bohart, 1975), and *Caesalpinia eriostachys* and *Parkinsonia aculeata* (Kevan, 1983). Nectar guides are described.

c) *Prosopis* (Simpson et al., 1977). The UV reflecting anthers are cited as near orientation cues.

d) *Cercidium floridum* and *C. microphyllum* (Jones, 1978). Differences in UV patterns act as effective prepollination isolating mechanisms between these two sympatric species.

e) *Lupinus arizonicus* and *L. sparsiflorus* (Wainwright, 1978). The nectar guide on the standard is not UV reflective either before or after pollination, but the petal is strongly UV absorbing against the background. This becomes indistinguishable after pollination.

f) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). The nectar guide is a yellow patch on a red background.

g) *Caragana arborescens* (Primack, 1982). UV patterns in the flowers are discussed.

h) *Astragalus, Vicia,* and *Wisteria* (Tanaka, 1982). The relationship between UV and visual spectral guidemarks is discussed in five species.

i) *Chamaecytisus palmensis* (Webb & Shand, 1985). The flowers are all white with no guidemarks under UV or daylight.

j) Various (Kay, 1987). This is a review of the occurrence of UV patterning in the Leguminosae. Unrelated, sympatric, yellow-flowered legumes with flowers that are indistinguishable to the human eye often show considerable diversity in UV patterning, which might promote pollinator constancy. Some genera, however (e.g., *Genista* and *Ononis*), show surprising uniformity in UV pattern, and this may extend to unrelated species that share the same pollinator (e.g., *Calicotome, Teline,* and *Ulex*). In the Australian Mirbelieae and Bossiaeeae, extreme examples of similar patterns occur in groups sharing the same pollinators. UV patterns can be due to different floral pigments (even in similar plants); for example, various mixtures of pigments can be found in the UV patterns of closely related species of *Coronilla* and in *Cytisus* and *Spartium*. UV absorbing patch markings or whole petals that may be interpreted as guidemarks are conspicuous in yellow zygomorphic flowers of the Caesalpinioideae and Papilionoideae; that is, the banner in many of the former and the standard in the latter are UV absorbant. In European and Mediterranean papilionoids (e.g., *Genista* and *Ulex*) the standard is often largely UV reflecting and contrasting with the UV absorbing wings and keel. A small patch, or UV absorbant guide, may be present at the base of the standard. In yellow papilionoids, complex UV patterns occur, involving radiating veins from the basal UV absorbing

guidemark; and, together with patterning on the wings and keel, this is a relatively precise cue for pollinator positioning and the operation of the tripping mechanism. Most ornithophilous papilionoids, unlike mellitophilous species, lack UV patterns (e.g., *Erythrina* and *Sophora*). In many Papilionoideae the visible guidemark coincides with or is only slightly different to the UV absorbing guidemark.

k) *Bauhinia monandra* and *Vicia sylvatica* (G. P. Lewis, pers. comm.) are species with distinct and colorful nectar guides.

5. Color changes: to becoming attractive over time (prepollination), increasing attraction due to pollinator availability, e.g., *Oxytropis* and *Lupinus nanus* (Kevan, 1983). Flowers in the first species change color from pink to blue over time, and the older blue flowers are visited more frequently by pollinators. Horovitz & Harding (cited in Kevan, 1983) showed how floral guides develop seasonally in *L. nanus*, increasing the plants attractiveness as pollinators become more frequent.

6. Extended attractive coloration: this occurs, for example, when a distinct (early) male phase is followed by stigmatic receptivity, then by color changes to become less attractive, thus signaling the end of the rewards. *Lupinus texensis* (Schaal & Leverich, 1980). Flowers open for up to six days, and the standard has a central white spot on a blue background. The first three days are a male-only phase, followed by peak stigma receptivity at day five. At this stage the spot turns red-purple and loses its attractiveness.

7. Pollen or nectar color as an attractant.

8. Color mimicry: see under floral mimesis.

G. Tactile cues

1. Petal appendages: hooks, rugosities, or callosities; spurs and the possibility of these being glandular and secretory. Any appendages might be part of the interlocking structure between the keel and wing petals, and they play an important role in the tripping mechanisms of many papilionoid legumes (see under deterrents and defenses). Appendages support grasping insects (spurs and sticky secretions [Nair & Tewari, 1975; Tewari & Nair, 1978a]) or cause petals to bulge, making a platform for pollinators to land (Stirton, 1977).

a) *Phaseolus mungo* and *Indigofera enneaphylla* (Nair & Tewari, 1975). Petal spurs are glandular at the tips.

b) *Canavalia virescens* (Stirton, 1977) and *Cyclopia aurescens* (Stirton, 1981a). Thickening and puckering of the margins of the wing petals is found in *Canavalia virescens*, and thickening and a deep depression in the area of the auricle occurs in *Cyclopia aurescens*. These features provide variation in the surface relief of the petal.

2. Sculpturing

a) Petal sculpturing: wings with pouch-shaped pockets (cavae), vacuolar cavities, and transversely arranged tissue bands (costae). These are associated with pollinator footholds and might be guidance cues. Variability in the position, arrangement, and shape of these features enhances flower recognition by pollinators. Epidermal microtexture changes along the petal might act as "nectar guides" (Kevan & Lane, 1985).

(1) *Crotalaria* (Tewari & Nair, 1978a). Wing morphology is described in ten species.

(2) *Cajanus cajan, Psoralea corylifolia, Sesbania bispinosa, S. sesban,* and *Tephrosia purpurea* (Tewari & Nair, 1979). Radial bands of tissue occur on the wings of *C. cajan*, and a sweet secretion is produced as an attractant for visitors. Tissue bands also occur in *P. corylifolia, S. bispinosa,* and *T. purpurea*. Cavae in *S. sesban* are radially elongated, pouchlike structures. Good descriptions of the wing morphology are given for the species.

(3) Various (Stirton, 1981a). A broad survey of petal sculpturing in genera and species of papilionoid legumes is given.

(4) Genistinae (Adey, 1982). Petal surface ornamentation characters are discussed.

b) Staminal sculpturing: surface sculpturing around the fenestrae (at the basal knee of the vexillary stamen) might be associated with tactile guidance of insect proboscises toward the nectary. In *Eriosema* (Stirton, 1981b), *Crotalaria graminicola*, and *Otholobium* (Stirton, pers. comm.), sculpturing occurs around the fenestrae.

3. Vestiture: functioning as tactile guides, that is, the presence and patterns of hairs on the petals (beards), papillae, or filaments with hairy bases.

a) *Jacqueshuberia quinquangulata* and *Melanoxylon* (Polhill & Vidal, 1981). The stamen filaments are hairy.

b) *Crotalaria graminicola* (Stirton, pers. comm.). Hairs occur around the auricles and the top of the claw at the base of the standard. The basal calluses and the inside of the claws are frequently shortly hairy in *Crotalaria* (Polhill, pers. comm.).

H. Flower odor

General references: Macior, 1971; Williams, 1983; Barth, 1985; Richards, 1986.

1. Flowers or individual petals

a) Localization and pattern of osmophores: producing volatile odors as attractants; variability in a group of species and the enhancement of flower recognition by pollinators; any correspondence between osmophore patterns and color patterns.

(1) *Spartium* and *Lupinus* (Esau, 1965). Osmophores are located on the wings in the former and on the standard in the latter.

(2) *Medicago sativa* (Loper & Lapioli, 1971). Flowers produce a volatile odor, with ocimene as the principle component.

(3) *Prosopis* (Simpson et al., 1977). Dense inflorescences of flowers with a sweet odor.

(4) *Cercidium floridum* and *C. microphyllum* (Jones, 1978). Osmophores are confined to the upper three stamens in the former and on the upper seven stamens, the claw, and scattered over the lateral petals in the latter.

(5) *Parkia javanica* (Wee & Rao, 1980) and *Parkia* sp. (Hopkins, 1983, 1984). These bat-pollinated inflorescences produce a foetid odor, and the flowers emit a strong sickly odor of overripe fruit as they open. This is also evident but weaker in the bud stage.

(6) *Pachyelasma* (Polhill & Vidal, 1981). This has red, malodorous flowers.

(7) *Erythrina batolobium* (Hernández & Toledo, 1982). A faint odor is recorded in this bird-pollinated flower.

(8) Genistinae (Adey, 1982). Regions of osmophore activity ("duftmale" patterns) are illustrated in a number of different species, the patterns all discernibly different. Differences in the amount of stain taken up indicate possible areas of greater scent production. In all species examined, the position of osmophore activity corresponded to some extent to the visual patterns.

(9) *Acacia* (Bernhardt, 1983). Inflorescence color tends to be relatively uniform in SE Australia, but floral fragrance is very variable between species. Odor is produced by the stigma and epidermis of the anthers but also by the filaments and petal margins in *A. myrtifolia*.

(10) *Inga* (Koptur, 1983). Six of seven species discussed produce a sweet scent, the other is yeasty. Information is given on the phenology of odor production.

(11) *Stylosanthes* (Pereira-Noronha et al., 1983). A faint odor is produced from the base of the standard petal.

(12) *Acacia retinodes* (Bernhardt et al., 1984). A distinctive floral fragrance like that

of a ripe cantaloupe melon is produced by the stigma and epidermal cells of the anthers. The odor is detectable during the female phase (flowers are protogynous) but is more pronounced during the male phase.

(13) *Bauhinia ungulata* (Ramirez et al., 1984). Flowers have a strong smell of rotting fruit late in the evening and are attractive to bats.

(14) *Chamaecytisus palmensis* (Webb & Shand, 1985). A faint odor is recorded from the flowers.

(15) *Anadenanthera colubrina, Sclerolobium,* and *Copaifera pubiflora* (G. P. Lewis, pers. comm.). In closely related genera of similar vegetation types, legume trees may partition pollinators by the intensities of odor production over time rather than by using different odors. These species are highly odoriferous, and the odors of *Anadenanthera* spp. and *Acacia* spp. are somewhat similar.

(16) *Ceratonia siliqua* (G. P. Lewis, pers. comm.). Antherodes (reduced anthers) in the female flowers produce an odor that is a weaker form of that produced by the male flowers.

(17) *Calliandra* (G. P. Lewis, pers. comm.). A change in attractants is found in some species. Species with banded red and white stamens attract visually during the day but have no or a very faint scent, while a strong scent is produced at night.

b) Time of production and intensity: specific times or different intensities of odor production may be associated with the attraction of particular types of pollinator (examples as above).

2. Pollen odor: presence of attracting chemicals, and any association with pre-imaginal conditioning in bees (Thorp, 1979).

a) *Caesalpinia eriostachys* and *Parkinsonia aculeata* (Jones & Buchmann, 1974). Some bees appear to be attracted to flowers based solely on their pollen odor.

b) *Cassia* (Buchmann, 1983). A pollen odor was noted.

c) *Desmodium incanum* (Schrire, 1984). Pollen develops a distinct "cucumber" smell in the morning hours when pollination is most likely to occur.

3. Nectar odor. *Inga vera* (Baker & Baker, 1982) produces sucrose-dominated nectar in the late afternoon and is visited by hummingbirds, various bees, wasps, and moths. About four hours later the nectar turns sourly odoriferous and is taken by bats.

4. Pheromones and any association between floral odors and similar substances produced by insects. *Cassia fistula* (Harborne, 1982): flowers produce a volatile similar to the pheromone of the fruit fly *Dacus dorsalis.*

I. Gustatory attraction

1. Phagostimulants in the pollen or nectar (e.g., pollen lipids and bees [Lepage & Boch, 1968; Hopkins et al., 1969]).

2. Volatiles from glands or trichomes.

J. Change in the type of attractants used with time

XI. DETERRENT AND DEFENSIVE FEATURES AGAINST OPPORTUNISTS OR HERBIVORES AND FOR PROTECTION OF RESOURCES

General references: Feeny, 1975; Levin, 1976; Faegri & van der Pijl, 1979; McKey, 1979; Rhoades, 1979; Rosenthal & Janzen, 1979; Price et al., 1980; Guerrant & Fiedler, 1981; Janzen,

1981; Polhill & Raven, 1981; Harborne, 1982; Gori, 1983; Barth, 1985; Duffey, 1986; Richards, 1986; Rosenthal, 1986.

Deterrent and defensive features may be physical structures or chemical compounds. Physical structures may abrade, puncture, or impede access to movement and feeding. Chemical compounds may be toxic, repellent or anutritive, and effective by contact, inhalation, or ingestion.

A. Inflorescences

1. Position: placed relatively obscurely and not projecting beyond the foliage, or the inflorescences are held well away from the foliage, deterring potential predators. *Parkia* (Hopkins, 1984): inflorescences are held out further from the crown in the neotropical species (sometimes up to 5 m) than in the paleotropical ones. Some are flagelliflorous, which might indicate an avoidance of predation by nonflying mammals.

2. Color: blending with the background (i.e., colors are inconspicuous against the foliage by day, and the inflorescences are attractive at night). *Hymenaea courbaril* (Arroyo, 1981): the greenish white flowers are borne in flat-topped racemes only slightly projecting above the subtending foliage. This avoids attraction during the day, but flowers produce nectar and attract bats at night.

B. Accessory organs limiting flower access

1. Spiny organs: spiny branchlets or brachyblasts, spiny stipules, thorns, prickles, persistent spiny leaf rachises, and spiny leaves, bracts, bracteoles, or calyx lobes (mechanical protection and any association with the shape and robustness of the plant).

   a) *Aspalathus* (Dahlgren, 1963), *Daviesia* (Carlquist, 1974), *Erinacea* (Stirton, pers. comm.). Examples with spiny branchlets or brachyblasts.

   b) *Gummifera* group of *Acacia* (Robbertse, 1974). Presence of spinescent stipules.

   c) *Genista tridentata* (Bisby, 1981). Presence of spinescent stipules.

   d) *Erythrina batolobium* (Hernández & Toledo, 1982). Sharp spines cover the peduncles, possibly preventing the Mexican flower piercer bird from robbing nectar, since it is unable to perch. This is also important in the switch from passerine to hummingbird pollination.

   e) *Vulgares* group of *Acacia* (Robbertse, 1974). Presence of scattered prickles.

   f) *Caesalpinia* group (Polhill & Vidal, 1981). Thorns and prickles are common.

   g) *Astragalus* (G. P. Lewis, pers. comm.). Presence of persistent spiny leaf rachises.

   h) *Ulex* (Bisby, 1981). Presence of spiny phyllodes.

   i) *Anarthrophyllum* (Stirton, pers. comm.). Presence of spiny-tipped leaves.

2. Covering, camouflage, and protection of the flowers by leaves, bracts, or bracteoles.

   a) *Psoralea imbricata* and *Geissaspis* (Stirton, pers. comm.) Flowers are often concealed in the axils of the leaves.

   b) *Desmodium longifolium* and *D. pulchellum* (Schrire, pers. obs.). The bracts are trifoliolate, foliacious, and almost completely conceal the flowers.

   c) *Zornia* (G. P. Lewis, pers. comm.). Large bracts with scattered glands occur in many species and they often almost completely cover the flowers.

   d) *Dalhousiea africana* (Polhill, 1981b). The bracteoles are large, enveloping the bud and partially covering the flowers.

   e) *Brownea* (Cowan & Polhill, 1981a). Flowers are clustered into large pincushion-like inflorescences, and the bracteoles are fused into long tubes around the base of each flower.

   f) *Podalyria calyptrata* (Stirton, pers. comm.). The young flower bud is protected by a large bractlike hood until it expands.

3. Hairs or glandular trichomes

a) Sticky or mucilaginous morphological barriers (moats) obstructing small insect movement into the flowers. *Stylosanthes* (Pereira-Noronha et al., 1983), *Bolusafra bituminosa*, and *Rhynchosia bullata* (Stirton, pers. comm.): glandular hairs occur on the bracts surrounding the capitular inflorescences. In *Bolusafra* and *Rhynchosia* the inflorescences, calyces, bracteoles, and bracts are sticky.

b) Stinging, or secreting toxic or slippery substances (see under chemical deterrents to herbivores). *Mucuna pruriens* (Lubis et al., 1977): stinging hairs occur on the calyx of forma *pruriens*.

C. Flowers

1. Size and shape restricting access of particular visitors to nectar or pollen, and any selection for the development of the trophic apparatus of pollinators.

a) *Trifolium nigrescens, T. michelianum, T. isthmocarpum*, and *T. xerocephalum* (Weaver & Weihing, 1960). Several clovers were very attractive to bees and yielded nectar profusely. The Persian clover *T. xerocephalum*, however, yielded less nectar, but its long corolla possibly reduces its attractiveness.

b) *Trifolium pratense* and *Melilotus alba* (Hobbs et al., 1961). The importance of tongue length in relation to flower size is stressed; long-tongued bumblebees prefer the former species and shorter-tongued bees prefer the latter.

c) *Erythrina oliviae* (Toledo & Hernández, 1979). The compact concavity for nectar, formed by the reduced keel and wings, limits accessibility to birds with elongated beaks.

d) *Anthyllis vulneraria* and *Onobrychis viciifolia* (Lack, 1982). The flower shape in the former makes nectar inaccessible to many *Bombus* pollinators. It attracts longer-tongued species.

e) *Erythrina batolobium* (Hernández & Toledo, 1982). The reproductive organs are in positions that are only likely to have contact with certain visitors. *Bombus* spp. approached the flowers for a short time, but they were doubtful pollinators, since contact with the reproductive organs was unlikely.

f) *Hardenbergia* and *Chorizema* (Stirton, pers. comm.). The association of flexible pedicels, wing petals much longer than the keel and with a distinctive lamellate surface sculpturing, and retraction of the androecium into the flower makes access difficult for bees. The pollinator has to be able to grasp the flower and dig in its head in order to operate the tripping mechanism.

2. Pollination (tripping) mechanisms (Arroyo, 1981) and the degree of floral complexity or difficulty to the pollinator in operating the flower mechanism to obtain the rewards.

a) Asymmetry or torsion of parts, restricting entry to the flower.

(1) *Vigna* (Lush, 1980) and *Bolusia* (Stirton, pers. comm.). The twisted keel in some species requires specific pollinators to operate the tripping mechanism.

(2) *Apios americana, Physostigma mesoponticum*, and *Phaseolus coccineus* (Lackey, 1981), *Vigna vexillata* (Hedstrom & Thulin, 1986), and *Vigna caracalla* (Arroyo, 1981). Species with varying degrees of twisting or spiraling of the keel.

b) Specialized or difficult tripping mechanisms: for example, explosive, valvular, or pump mechanisms.

(1) *Medicago sativa* (Pedersen & Bohart, 1953; Hobbs et al., 1961; Akerberg, 1967). Many bees find the tripping mechanism difficult to operate, and they either approach from the side or drill a hole through the calyx to the nectary.

(2) *Indigofera spicata, Phaseolus lathyroides, Desmodium uncinatum*, and *Stylosanthes gracilis* (Hutton, 1960). Species with explosive tripping mechanisms.

(3) *Colutea arborescens* (Nye & Bohart, 1962). The mechanism is difficult to operate and can only be worked successfully by "heavy insects."

(4) *Alysicarpus vaginalis* and *Desmodium* (Chow, 1974). The explosive floral tripping mechanism is described.

(5) *Mucuna andreana* (Frankie & Baker, 1974). Explosive opening of flowers is brought about by pollinating bats.

(6) *Eriosema* (Stirton, 1975). The valvular tripping mechanisms of the flowers is discussed.

(7) *Canavalia virosa* (Stirton, 1977). The complex tripping mechanism is discussed. (See c, below.)

(8) Genistinae (Adey, 1978). Bees smaller than the typical pollinators (*Xylocopa* or *Apis*) trigger the floral mechanism by pure persistence, forcing apart the keel petals with their head, mouthparts, and front legs.

(9) *Erythrina fusca* (Feinsinger et al., 1979). The standard must spring back to expose the anthers and stigma, and this requires the pressure of a foraging bird.

(10) *Ulex europaeus, Cytisus scoparius,* and *Medicago sativa* (Meeuse, 1979). The tripping mechanisms are described.

(11) Papilionoideae (Arroyo, 1981). Information is given at the tribal level of the occurence of pump, brush, and explosive tripping mechanisms.

(12) *Cytisus scoparius* (Bisby, 1981). When the flowers trip eruptively, the spring-loaded style and stamens hit the bee and recoil.

(13) *Anthyllis vulneraria* and *Onobrychis viciifolia* (Lack, 1982). No male *Bombus* visitors came to the flowers; they do not collect for the nest and were seen avoiding flowers where access was difficult.

(14) Genistinae (Adey, 1982). Classes of bee based on body weight are associated with classes of flower based on structural and size characters. A strong relationship was found between bee type and flower type in the 27 species examined.

(15) *Stylosanthes* (Pereira-Noronha et al., 1983). The pump tripping mechanism is similar to that in *Arachis* (Periasamy & Sampoornam, 1984), with prolongation of the five internal stamens in a hypanthial tube. Hooks on the petals are associated with petal configuration and the tripping mechanism.

(16) *Lupinus palestinus* and *L. pilosus* (Pazy, 1984). Self-pollination is the rule, but flowers have to be tripped by insects.

(17) *Chamaecytisus palmensis* (Webb & Shand, 1985). Only *Bombus terristris* successfully gained access to the flowers, which require considerable force to operate.

(18) *Lotus corniculatus* (Jones et al., 1986). When the wing petals are depressed, the keel (and dark keel tip) are revealed. The wings slowly return to their original position after ± 15 minutes. Many visits are required for a full seed set.

(19) *Vigna vexillata* (Hedstrom & Thulin, 1986). The weight and pressure of the pollinator, *Xylocopa gualanensis*, on the left-hand wing and keel petal in the assymetrical flower causes the upper bearded part of the style, along with the upper free parts of the stamens, to slip out of the rigid keel beak, "hugging" the bee over the dorsal part of its head and thorax.

c) Resupination and an increase in the difficulty of access to the flower. A transfer of function occurs from the wings and keel to the standard, which now acts as a landing platform; this requires specialized behavior in the pollinators to gain access to the rewards.

(1) *Canavalia virosa* (Stirton, 1977). The standard is inverted and faces upward, forming a landing platform for the pollinator, *Xylocopa flavorufa*. Many such flowers are rigidly constructed to allow access to large, high energy–demanding pollinators. The mechanism is so arranged that the insect digs its head into an opening between the wings and is dusted on its back with pollen. The mechanism returns to its original position afterwards. If the pollinator is not a perfect fit, as for example

with *Megachile combusta,* it positions itself in the opposite direction, with its abdomen over the tip of the keel. This bee is more of an opportunist.

(2) *Centrosema pubescens, Clitoria ternatea,* and *Canavalia rosea* (Lackey, 1981). Examples of species with resupinate flowers.

3. Flower position restricting access to particular visitors or requiring specialized behavior to obtain rewards.

a) *Sophora* and *Clianthus* (McCann, 1952). In the New Zealand species, the flower pedicels are versatile, associated with perching birds that bend their heads down and, using the standard as a lever, swivel the flowers upward into an appropriate position for nectar extraction.

b) *Stylosanthes* (Pereira-Noronha et al., 1983). Flowers are aggregated in dense capitular inflorescences, surrounded by bracts and glandular hairs.

c) *Trifolium repens* (Lloyd & Webb, 1986). Reflexion of senescent flowers make others in the capitulum more accessible.

4. Flower color

a) *Cupulanthus* (Keighery, 1982). Flowers are a dull brown-red, and although they have a bird-pollination syndrome, the species is seldom visited by birds. It is autogamous and habitually selfing.

b) *Anthyllis vulneraria* and *Onobrychis viciifolia* (Lack, 1982). The two species had a 73% overlap in flowering time, yet the proportion of similarity of *Bombus* spp. visitors was only 20%. The former has yellow flowers, the latter pink flowers. This was also due to *A. vulneraria* having inaccessible nectar and attracting longer-tongued species.

5. Flower entrance blocked by a plug of hairs or bristles.

D. Individual floral parts: size, shape, number, position, or color limiting or deterring access to the flower.

1. Calyx

a) *Caesalpinia* group (Polhill & Vidal, 1981). The lowermost sepal is more or less boat-shaped and quite often forms a hood in bud.

b) *Alexa grandiflora* (Stirton, pers. comm.). The large woody calyx is rich in sclereids and protects the buds. It is persistent and protects the fleshy hypanthium.

2. Corolla

a) Shape and structure

(1) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). The claw of the banner petal is modified into a narrow tube, restricting access to the nectaries.

(2) *Erythrina pallida, E. fusca,* and *E. poeppigiana* (Feinsinger et al., 1979). The energy output per flower in terms of nectar is high. Extrafloral nectaries attract ants, but floral nectar is protected from them by the waxy inner surface of the petals. The two spatially proximal nectaries are thus kept separate.

(3) *Eperua* (Arroyo, 1981). The single petal is modified into a hoodlike structure partially enclosing the stamens. This is part of the specialized mechanism for bat pollination.

(4) *Crotalaria* and Phaseoleae (Polhill, pers. comm.). Appendages at the base of the standard in many of these species limit access to the nectaries.

b) Cryptic coloration of petals blending with the background, and the flowers either become attractive only at night or they limit the range of pollinators by restricting attraction to only one petal. *Afzelia quanzensis* (G. P. Lewis, pers. comm.): the floral parts are green, with only the banner petal red, this possibly not visible to many potential visitors.

c) Color and UV patterns and contrasts—combined with distinctive osmophore patterns and different odors, various tactile cues, and various rewards—act as isolating mechanisms, se-

lecting for pollinator fidelity and deterring opportunists. Keel petal tip coloration may be associated with a concentration of deterrents against herbivory, or it may be a cryptic feature hidden by the wings until the flower is tripped (Jones et al., 1986).

3. Androecium

a) Degree of division of labor among stamens (staminodes or functionally different stamens) and any selection for correctness of fit of the pollinator, with an optimum placement of reproductive pollen on its body. *Parkia* (Hopkins, 1984): the staminodal fringe in the capitular inflorescences is sometimes well developed and overhangs the nectariferous flowers. It might protect nectar from dilution by rain (staminodes are water-repellent) or reduce nectar evaporation. The staminodes also act as physical barriers to small nectar thieves and as a landing platform for bats to clasp the capitulum.

b) Cryptic polliniferous anthers and/or "pseudopollen" decoys.

c) Anther glands or tufts of hairs: in a defensive role against predation, the anthers may be protected by a hood or the hairs may be associated with separating the stigma from self-pollen.

(1) *Martiodendron* (Koeppen & Iltis, 1962). Anthers dehisce by two subterminal pores. They are covered by a hygroscopic hoodlike terminal pollen cup, which when moistened closes laterally to cover the pores. When dry, they twist approximately 90°, which places the pollen cup beneath the anther pores. This also plays a role in promoting pollen transfer.

(2) Parkieae, Mimoseae, and Acacieae (Elias, 1981). The anthers are frequently gland-tipped.

(3) Indigoferae (Polhill, 1981a). The anthers are either gland-tipped or are expanded with anther appendages. These might be more important as attractants and in pollen transfer to the vector (Schrire, pers. obs.).

(4) *Harleyodendron* (Cowan, 1981). The anthers are coherent by intertwined hairs.

(5) *Mora* (Polhill & Vidal, 1981). The anthers are woolly at the apex.

d) Protection of the nectaries from opportunists and herbivores and to prevent drying out or dilution of nectar (due to wind, rain, or solar radiation). They may be hidden in position. covered by a fringe of staminodes (Hopkins, 1984), or by a fringe of hairs, or protected by flower movement (e.g., closure in bad weather and at night) or be protected by the outer whorls. The degree of stamen fusion limiting access to the nectaries (i.e., diadelphy developing either to monadelphy or pseudo-monadelphy; Tucker, this volume). The vexillary stamen may be adherent or adnate to the others, or with a basal knee associated with the presence of basal fenestrae and the development or loss of nectaries.

4. Gynoecium

a) Enantiostyly: style shape as a possible adaptation to avoid damage to the minute stigma during pollinator vibration (Buchmann, 1983).

E. Postpollination changes

1. Positional: pollinator efficiency may be enhanced by preventing nonproductive visits by pollinators, for example, flower deflexion or floral part movement altering the symmetry of the flower and blocking the attractive cues.

a) *Alysicarpus vaginalis* and *Desmodium* (Chow, 1974). The tripped flower wilts quickly with the standard folding over the stigma.

b) *Caesalpinia eriostachys* (Jones & Buchmann, 1974). The banner petal folds down over the stamens, and although the absorptive UV pattern is unaffected, the floral symmetry changes.

c) *Cercidium floridum* (Gori, 1983). The banner petal folds over the stigma and style.

d) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). Positional changes in the floral parts were noted, making the flowers unattractive to pollinators.

e) *Indigofera* (Polhill, 1981a). The petals are nearly always caducous after the flower has explosively tripped.

2. Color: visual and UV color changes may alter contrasts and cause loss of attractiveness (and might signal the end of the rewards).

a) *Parkinsonia aculeata* and *Caesalpinia eriostachys* (Jones & Buchmann, 1974). Morphological and physiological changes occur in the floral color patterns of both species following pollination; for example, the banner petal in *P. aculeata* becomes darker orange and is not visited by bees.

b) *Lupinus arizonicus* and *L. sparsiflorus* (Wainwright, 1978). Distinct postpollination color changes occur to the nectar guide on the standard, and this is associated with avoidance by pollinators. The change is a response to flower manipulation during the receptive stage of the stigma and is not a result of fertilization.

c) *Caesalpinia pulcherrima* (Cruden & Hermann-Parker, 1979). The flower color becomes uniformly red after pollination.

d) *Senna didymobotrya* (Dulberger, 1981). Color changes occur in the two pollinating anthers, from yellow to dark brown, later in the day.

e) *Lupinus argenteus, L. texensis, Daviesia pectinata, Hovea trisperma, Argyrolobium rupestre, Caesalpinia pulcherrima, Cercidium microphyllum, Parkinsonia aculeata,* and *Bauhinia flammifera* (Gori, 1983). Postpollination color changes are noted.

f) *Lupinus texensis* and *Lotus scoparius* (Kevan, 1983). In the former, over 90% of bee visits were to flowers with a conspicuous white to yellow banner spot. This changes to red-purple after five days, when visitations cease, since no more contrast is presented. *Lotus scoparius* flowers are also described as changing from yellow to burnt orange.

g) *Delonix regia* and *Copaifera* (G. P. Lewis, pers. comm.). *Delonix regia* has a predominantly white banner petal which becomes suffused with red (matching the others) after pollination. This also occurs with age in the flower, so pollination may not be necessary for the color change. In *Copaifera* spp. the white filaments of the stamens change to pink after pollination.

h) *Desmodium* (Schrire, 1984). Flowers turn a uniform dull bluish after pollination.

F. Specific time of attractant or reward presentation: or time release mechanisms, for example, poricidal anther dehiscence limiting the presentation of rewards to a period when pollination is most likely to occur. Short flowering times and/or short periods of pollen viability or stigma receptivity limit potential visitors.

G. Control of nectar production. Controlling the quantity of nectar produced might influence pollinator movement; for example, large quantities of nectar might be associated with long visits because of high pollen carryover (Plowright & Hartling, 1981), or restricted amounts might promote greater interflower movement.

H. Strategic vegetative ploys: for example, hugging the ground and avoiding predation or damage (*Trifolium* in lawns [Stirton, pers. comm.]).

I. Palatability deterrents

1. Physical deterrents that reduce the plant's palatibility to herbivores.

a) Cell inclusions: the presence of calcium oxalate crystals, rhaphides, fibers, or sclereids in the tissues (woody bracts, bracteoles, and calyces in bud protection).

(1) *Lotus* (Solereder, 1899). Druse crystals were noted in the calyx.

(2) Various (Buss & Lersten, 1972; Zindler-Frank, 1987). Buss & Lersten surveyed calcium oxalate crystals found in the tapetal cells of all three subfamilies. These vary from small scattered crystals of various forms to larger lenslike or prismatic crystals. Styloid crystals occur in the Caesalpinioideae. A survey of the presence of calcium oxalate crystals in legumes (Zindler-Frank, 1987) showed that cells with these crystals are located below the bases of biramous hairs in *Indigofera lespedezioides*. Crystals in general appear to play a role in antiherbivory.

(3) *Crotalaria* (Tewari & Nair, 1978a). Sclereids are located in the distal (apical) parts of the petals.

(4) *Prosopis, Piptadenia, Inga, Calliandra, Affonsea, Cynometra, Dimorphandra, Ormosia,* and *Cassia* (Rao & Das, 1979). Various genera are cited with diffuse sclereids in the leaves. These should be sought for in the floral parts. *Cynometra* has idiofibrosclereids.

(5) *Sophora* (Crowder, 1982). Crystals were noted in the petals.

(6) *Alexa grandiflora* (Stirton, pers. comm.). The species has a persistent woody calyx rich in sclereids.

b) Vestiture, glands, scales, or cuticular deposits: the presence of hairs, trichomes, etc. on the inflorescence or flowers; plant trichomes and the lowering of foraging efficiency of herbivores by impeding movement and increasing search time, either physically or with the addition of sticky or viscous exudates. The presence of cuticular deposits (e.g., waxes) and a thick epidermis (sclerophylly).

(1) *Eminia* (Taubert, 1891). Conspicuous glands occur at the tips of the acuminate calyx lobes and produce a sticky secretion in the bud stage, protecting the young petals against herbivory.

(2) *Aspalathus* (Dahlgren, 1963). Various trichomes in the genus are discussed.

(3) *Eriosema* (Stirton, 1975). The back of the standard is nearly always pubescent and glandular. The other petals are also scattered, with hairs and glands.

(4) *Crotalaria* (Tewari & Nair, 1978a; Gupta, 1980). Hairs (both unicellular and bicellular) occur on the anterior and posterior parts of the petal, presenting a possible defense against chewing insects. Gupta discusses trichomes in 18 species. They occur mostly on the pedicels and calyces but occasionally on the standard, keel, gynoecium, and bracts. Seven kinds of nonglandular and two types of glandular trichomes are found.

(5) *Hymenaea* (Cowan & Polhill, 1981a). Presence of pustulate-glandular petals containing resin.

(6) *Polystemonanthus* and *Dicymbe* (Cowan & Polhill, 1981b). Bracteoles with large terminal knoblike glands.

(7) *Phaseoleae* (Lackey, 1981). Trichomes and glands are discussed.

(8) *Caesalpinia* group (Polhill & Vidal, 1981). Scattered glands and glandular hairs are common in this group.

(9) *Medicago scutellata* (Kreitner & Sorensen, 1983). Erect capitate stalked glands are widespread on the shoot apices, stems, and leaves, and they produce a viscous lipophilic secretion shown to be effective in immobilizing larvae of alfalfa weevil.

(10) *Bauhinia* (Tucker et al., 1984). Inflated bifid multicellular trichomes occur on the sepals, petals, stamens, and carpels (including the rest of the plant) in 11 species, but they do not occur on all organs in each species. Secretory products are given.

(11) *Tephrosia, Mundulea, Alexa,* and *Luetzelburgia* (Stirton, pers. comm.). Hairs on the petals are common.

(12) *Dolichos* (Stirton, pers. comm.). Densely packed short tubular glands occur along the upper margins of the keel petals.

(13) *Stryphnodendron rotundifolium* (G. P. Lewis, pers. comm.). Overlapping brown scales cover the inflorescences and bracts.

2. Chemical deterrents to herbivores: broad spectrum deterrents or specific toxins such as herbivore metabolic blockers, insecticides, growth inhibitors (e.g., in herbivore larvae), antihormones or hormone analogues, abortifacients or substances interfering with the reproductive capacity of herbivores or with insect metamorphosis and development, herbivore digestion inhibitors (protease inhibitors or tannins), mimics of herbivore alarm pheromones, allergenics, allelopathic substances to phytotoxins and pathogens, blood coagulents, neurotoxins, and inducible defenses. Inducible defenses may occur through pheromonal communication between plants, e.g., the warning systems among various leguminous trees and temporary increases in the levels of tannins as a response to browsing pressure (Van Hoven, 1984).

a) Secretion of toxic or slippery substances from the trichomes or glands.

b) Accumulation of unpleasant or toxic secondary compounds.

(1) Nonprotein amino acids (Bell, 1981).

(a) *Astragalus, Vicia sativa, Lathyrus, Leucena leucocephala,* and *Mimosa* (Levin, 1976); *Indigofera spicata, Mimosa pudica, Leucaena leucocephala, Astragalus* sp., and *Neptunia amplexicaulis* (Rosenthal & Bell, 1979). Various toxic amino acids are discussed.

(b) *Astragalus racemosus* (Harborne, 1982). An example of a species accumulating selenium amino acids.

(2) Terpenoids

(a) Saponins: various (Applebaum & Birk, 1979). Saponins occur in the roots, foliage, and flowers of a number of forage legumes, and their inhibitory or toxic effects are discussed.

(b) Resins (Langenheim et al., 1979; Langenheim, 1981). *Hymenaea* (Langenheim et al., 1979; Price et al., 1980; Cowan & Polhill, 1981a). The petals are glandular pustulate and contain resin. Armyworm larvae were increasingly subject to mortality from viral attack after being fed resin. *Dalea* and Amorpheae (Turner, 1986) and Psoraleeae [African] (Boardley et al., 1986): droplets of resin were found on the leaf surfaces above cavities found along the leaf margins and the midrib, where chewing insects are most likely to feed.

(3) Phenolics (e.g., tannins). *Lathyrus pratensis* and *Lespedeza cuneata* (Swain, 1979); the palatibility of these is dependent on the tannin content. *Inga densiflora* and *I. punctata* (Koptur, 1985): upland populations of these species of *Inga* have higher concentrations of phenolics in their foliage than lowland populations; this is associated with, among other aspects, a switch in herbivore defenses between populations at either end of the altitude gradient.

(4) Cyanogenic glycosides, including glucosides of organic nitro compounds (Jones, 1974; Harborne, 1982; Seigler et al., this volume).

(a) Various (Conn, 1979). One hundred twenty-five species of legumes are listed for producing cyanide when the tissues are crushed.

(b) Various non-ant acacias (Janzen, 1981). Trends for cyanide production occur in these, but generally not in ant-acacias.

(c) *Lotus corniculatus* (Compton & Jones, 1985; Rosenthal, 1986). The flowers support an extensive insect community of seed, pod, pollen, and petal feeding species. Some insects made no distinction between cyanogenic and acyanogenic plants (e.g., beetle larvae), but many others (e.g., grasshoppers) avoided cyanogenic petals completely.

(d) *Astragalus* (Harborne, 1982). Occurrence of miserotoxin, a glucoside of an organic nitro compound.

(e) *Indigofera* and some *Lonchocarpus* produce the glucoside indican, which might be a herbivore deterrent (Schrire, pers. obs.).

(5) Alkaloids: quinolizidine or pyrrolizidine (Smolenski & Kinghorn, 1981; Kinghorn & Smolenski, 1981; Harborne, 1982).

(a) *Crotalaria* (Levin, 1976). Pyrrolizidine alkaloids are stored and sequestered by *Amphicallia*, a herbivore that feeds on these plants. The alkaloids are used as a defense by the herbivore.

(b) *Lupinus* (Rhoades, 1979). High intraspecific qualitative and quantitative variation occurs in the alkaloid profile of the inflorescences in populations exposed to high potential herbivore pressure. This was noted in those populations growing under long flowering seasons in moist sites, but not in dry sites where herbivore pressure is low.

(c) *Lupinus* (Wink, 1985). Compared with alkaloid-free plants, the presence of quinolizidine alkaloids deters feeding by herbivores.

(6) Flavonoids: particularly isoflavonoids and their effect on oestrogenic activity in mammals, or as effective deterrents to feeding insect larvae (Sutherland et al., 1980). Any possible association with disease resistance in legumes?

(a) *Genista lydia* (Ulubelen & Doguc, 1974). Flavonoids in the flowers are discussed.

(b) *Derris*, *Lonchocarpus*, *Mundulea*, and *Tephrosia* (Harborne, 1979). Isoflavonoids, particularly rotenoids, acting as insecticides are discussed. Flavonoids include a number of floral color pigments. Various colorless flavonoids might be feeding deterrents.

(c) Various isoflavonoids are discussed (Harborne, 1982), e.g., genistein and formononetin in *Trifolium subterraneum* and coumestrol in *Medicago sativa* and *Trifolium repens*.

(d) Millettieae (Menichini et al., 1982). The flavonoids and rotenoids occurring in this and related tribes are discussed.

(7) Phytoalexins derived from isoflavonoids, and any association with disease resistance in plants (Ingham, 1981).

(8) Proteinase inhibitors: inhibiting proteolytic enzymes (Ryan, 1979; Weder, 1981).

(9) Lectins: phytohaemagglutinins and a possible role as defense agents against pathogenic bacteria by protein recognition; also as binding agents for symbiotic bacteria (Liener, 1979; Toms, 1981).

(10) Mixed chemical defenses (Guerrant & Fiedler, 1981).

c) Poisonous nectar: variability in the intraspecific level of toxicity and community dynamics.

(1) *Sophora microphylla* (Clinch et al., 1972). Nectar frequently has a narcotic effect on honeybees; narcosis and mortality appear to be due to alkaloids reaching the nectaries. The plant produces copious nectar, which is very attractive, and the toxic elements do not act as a deterrent. Experiments have shown that the level of toxicity varies among trees, being high in some and apparently absent in others. Similar reports have been made from *Sophora japonica*.

(2) *Astragalus lentiginasus* (Baker & Baker, 1975). Nectar or honey from this species is toxic to various kinds of animals, including humans. Tests for the presence of alkaloids in the nectar were positive for *Lotus corniculatus*.

(3) *Astragalus miser* var. *serotinus* (Majak et al., 1980). Nectar contains miserotoxin, a nitropropanol glucoside. Feeding tests with caged bees demonstrated that such nitro compounds are toxic.

(4) *Inga oerstediana*, *Erythrina fusca*, and *Centrosema plumieri* (Guerrant & Fiedler, 1981). All three nectars contained nonprotein amino acids. *Erythrina fusca* had three potentially deterrent substances, as well as phenolics and alkaloids.

d) Phagorepellents in pollen.

e) Low nutritive quality of tissues.

J. Attraction and use of accessory organisms: for example, ants as defense mechanisms, and attraction by extrafloral nectaries (Bentley, 1976; Guerrant & Fiedler, 1981; McKey, this volume).

a) *Acacia* (Janzen, 1974). New World obligate acacia ants live in the swollen stipular thorns of the group known as "swollen thorn" acacias, which have large and partly hollow stipular thorns that the ants clean out through an entrance hole cut in one thorn tip while the thorn is still green and relatively soft. The plants also have greatly enlarged petiolar nectaries secreting nectar for the ants, as well as a year-round leaf production by most individuals, yielding a relatively continuous food source for the ants. They have lost most of the chemical and structural traits that protect other acacias from most herbivores. More of these species inhabit disturbed habitats in moister areas than many non-ant acacias. In addition to protection, ants give the plants a competitive ability to grow fast in these regions by pruning vines and killing intruding branches.

b) *Senna pallida* (Silander, 1978). Plant clumps are colonized by aggressive predatory ants, which visit extrafloral nectaries on the pedicels of flowers and on the leaves and show aggressive behavior toward insects. Significantly lower levels of herbivory were found on plants colonized by *Crematogaster*.

c) *Erythrina pallida, E. fusca,* and *E. poeppigiana* (Feinsinger et al., 1979). Extrafloral nectaries occur on the ventral lip of the calyces (and are especially pronounced in bud) and elsewhere on the inflorescences of all three species. Nectaries secrete glistening droplets of nectar, attracting belligerent ants. Trees with ants removed had significantly greater predation of inflorescences.

d) *Vicia* sp. (Koptur, 1979). Stipular nectaries are visited by the Argentine ant, and plants excised of nectaries suffered considerably greater damage from predation. These nectaries, however, also function temporally and spatially to attract pollinators to the flowers.

e) *Erythrina flabelliformis, E. abyssinica,* and *E. humeana* (Sherbrooke & Scheerens, 1979). Extrafloral nectaries occur on the calyx and leaf in the first two species but only on the calyx in *E. humeana*.

f) *Acacia, Tachigali,* and *Leonardoxa* are discussed by Janzen (1981) and McKey (this volume) as having obligate ant associations.

g) *Erythrina batolobium* (Hernández & Toledo, 1982). The absence of extrafloral nectaries might account for the large (±25%) level of flower predation by lepidopteran larvae.

h) *Pterocarpus amazonicum* (Stirton, pers. comm.; voucher: *Prance et al. 13741* [K]). The presence of swollen inflorescence axes inhabited by ants.

i) *Indigofera* (Schrire, pers. obs.). Small red multicellular glands in the leaf axils are frequently "milked" by ants.

K. Attraction of parasitoids: e.g., Hymenoptera and Diptera to parasitize herbivore larvae (by extrafloral nectaries [Koptur, 1985]).

L. Camouflaged ambush predators: e.g., arachnids in flowers (Morse, 1976; Simpson et al., 1977 on *Prosopis*), and possible selection for pollinator type.

M. Alternative defense strategies: e.g., with changes in altitude between populations of the same species (Koptur, 1985). Populations of two species of *Inga* were found to change their defenses from low to high altitude. Populations at lower elevation attract aggressive ants to extrafloral nectaries. Populations at a higher elevation retain actively secreting extrafloral nectaries, but because ants are less active, they attract hymenopteran and dipteran parasitoids to lepidopteran larvae (the principal herbivores

at both altitudes). In addition, upland populations have higher concentrations of phenolics in the foliage than low altitude populations. Despite this, herbivore damage to leaves was greater at higher altitudes, although the abundance of the herbivore larvae was no different.

N. Qualitative versus quantitative defenses

Qualitative defenses interfering with the internal metabolism of the herbivore are associated with ephemeral or rare plants and plant tissues; quantitative defenses that reduce the digestibility of plant tissues are associated with the most predictable and available plants and plant tissues.

O. Variability in levels of defense chemicals: this might be of advantage to high-risk populations in minimizing the chance of counteradaptation by herbivores.

P. Mixed defense strategies using chemical, physical, or biotic defenses, or any combination of these.

SEED:OVULE RATIOS, SELECTIVE SEED ABORTION, AND MATING SYSTEMS IN LEGUMINOSAE

K. S. Bawa and D. P. Buckley[1]

Abstract

In legumes, as in many other plants, only a small fraction of flowers mature into fruits. Also, within flowers, only a certain proportion of ovules develop into seeds. We examine the relationship of seed:ovule ratios and mating systems in the three subfamilies of Leguminosae. We show that seed:ovule ratios are uniformly higher in selfers or inbreeders than in outcrossers. Our data also indicate a nonrandom pattern of seed maturation within fruits in 13 out of 14 surveyed species. In particular, ovules closest to the stylar end of the ovary have the highest probability of developing into a mature seed in all but one of the thirteen species exhibiting nonrandom patterns of seed abortion. We interpret our data to mean that microgametophytic selection and uncertainty about the genetic quality of pollen arriving at the stigma are major factors in the abortion of flowers, fruits, and seeds. We ascribe the probable primary cause of nonrandom seed maturation to sorting of seed quality within fruits by microgametophytic selection, although we have not excluded alternative explanations in this study. Finally, we argue that a consideration of factors leading to variation in seed:ovule ratios and fruit:flower ratios is essential in understanding the evolution of many reproductive traits.

I. Introduction

Legumes, like many other outcrossing plants, bear many more flowers than mature fruits. Furthermore, within flowers of most species, there are many more ovules than those that are matured to seed (Bawa & Webb, 1984; Wiens, 1984). There is mounting evidence that some of the excess flowers and a large fraction of the extra ovules are borne to screen the genetic quality of the offspring (Janzen, 1977; Lloyd, 1979; Lee & Bazzaz, 1982a, b, 1986; Lee, 1984; Bawa & Webb, 1984; Weins, 1984). Below, we report a comparative survey of Costa Rican legume species sampled to evaluate the extent of variation in seed:ovule and fruit:flower ratios. Our objective is to test the hypotheses that (1) selective abortion of low quality embryos is an important cause of low seed:ovule ratios and that (2) seed:ovule ratio variation is correspondingly related to mating system variability.

We have chosen to study seed:ovule ratios in tropical legumes for several reasons. As a very diverse clade, Leguminosae provide a rich source of comparative data, with comparisons possible at several different taxonomic levels. Because legume diversity is high in the dry semideciduous forests of Guanacaste, Costa Rica, we are able to control somewhat for variation that might be imposed by phylogenetic constraints operating within different lineages or due to sampling species in very different environments. Where possible, we have employed sympatric, congeneric species pairs to make comparisons. Lastly, legumes exhibit reproductive attributes that we wanted to exploit in order to test the hypothesis that selective seed abortion is a widespread and significant cause of low seed:ovule ratios. Many legume species provide a relatively unique opportunity to study interactions between gametophyte selection and embryo selection because of (1) the linear arrangement of ovules within ovaries and (2) the limitations that mimosoid pollen units might impose on opportunities for gametophytic selection (Bawa & Webb, 1984; Webb & Bawa, 1985; Lee, 1984; Lee & Bazzaz, 1986).

Furthermore, we focus on seed abortion because it has received less attention than fruit abortion

[1]Department of Biology, University of Massachusetts, Boston, Massachusetts 02125, U.S.A

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has in previous research (see Stephenson, 1981; Bookman, 1984; Lee & Bazzaz, 1986; Nakamura, 1986; Temme, 1986). Selective seed and fruit abortion and microgametophytic selection might be functionally related because all three processes might represent complementary or alternative means of screening offspring quality before major maternal resource investment occurs. However, not all of the hypotheses explaining seed:ovule ratios have considered the implications of gametophytic selection carefully (e.g., Wiens, 1984; but see Lee, 1984). Others have not dealt with the relationship of seed abortion with mating system (e.g., Lee & Bazzaz, 1982b, 1986; but see Bawa & Webb, 1984).

Although we focus on seed abortion, we emphasize that factors underlying variation in seed:ovule ratios must be considered in conjunction with factors responsible for variation in fruit:flower ratios. We believe that many flowers that are successfully pollinated but fail to develop mature fruits substitute for selection among embryos within the developing fruits. Accordingly, we also present data on fruit:flower ratios along with seed:ovule ratios.

II. Hypotheses

Several related genetic hypotheses have been proposed to explain variation in seed:ovule ratios among species. Buchholz (1922), Janzen (1977), and Westoby & Rice (1982) have interpreted low seed:ovule ratios as a mechanism for maternal regulation of offspring genetic quality by selective abortion of less vigorous progeny. Wiens (1984) emphasized the possibility that seed abortion represents a mechanism for the selective elimination of genetic load. Bawa & Webb (1984) emphasized that variation in the genetic quality of pollen arriving on outcrossing species' stigmas is greater than that for selfing species and that increased seed abortion rates may have evolved in compensation for the higher degree of uncertainty about paternal genetic quality. Because uncertainty about pollen parentage is greatly reduced in structured or predominantly selfing populations, Bawa & Webb (1984) argued that selfers should exhibit higher seed:ovule ratios than outcrossers. To the extent that outcrossing results in genetic load, the two hypotheses are related. Outcrossers are expected to accumulate more recessive mutational load and to produce more segregational and other recombinational loads. However, selective seed abortion may also exist as a mate choice mechanism independent of genetic load, e.g., as a means of evaluating paternal specific combining ability, perhaps maintained by sexual selection (Bawa & Webb, 1984).

Wiens (1984) and Bawa & Webb (1984) differ in their emphasis on the influence of gametophytic selection on seed abortion position effects within fruits. Wiens (1984) assumes that seed abortions are randomly distributed within fruits. However, Bawa & Webb (1984) and Lee (1984) propose that microgametophytic selection may be capable of sorting the genetic quality of seed progeny within a fruit. To the extent that (1) different ovule positions tend to be fertilized by differentially vigorous microgametophytes and (2) genes expressed in both microgametophytes and sporophytes are positively correlated in vigor (Mulcahy et al., 1978; Mulcahy, 1979; Zamir, 1983; Bawa & Webb, 1984; Lee, 1984), then seed vigor (abortion rate) will be related to ovule position within fruits. For example, no position effect is expected if all ovules are equidistant from the point at which the pollen tubes enter the ovary. However, in fruits where fertilization is sequential and the sequence is morphologically discernible (e.g., many legumes), then seed abortion pattern can be used as a test for the hypothesis that seed abortions are selective (Bawa & Webb, 1984; and see below). This criterion must be applied with caution because other mechanisms can produce similar seed abortion patterns, as will be discussed below.

Predictions

Both Wiens (1984) and Bawa & Webb (1984) predict that (1) outcrossers should have lower seed:ovule ratios than selfers and (2) seed:ovule ratios less than unity reflect selective seed abortion.

at least to a significant degree. Wiens (1984) demonstrated that seed:ovule ratios appear to be more closely related to longevity than mating system; however, he did not consider the potentially complementary role that gametophyte selection and fruit selection can play, nor did he demonstrate that seed abortion is selective. Bawa & Webb (1984), Webb & Bawa (1985), and Lee & Bazzaz (1986) demonstrated patterns of seed abortion within fruit that are consistent with the interpretation that seed abortion rates are influenced by a preceding phase of microgametophytic selection, which, if true, indicates that seed abortions are selective too. However, all of their data were collected from outcrossing species, so no comparisons of seed:ovule ratios or patterns of seed abortion in differing mating systems could be made. The study reported here is designed to evaluate the relationship of seed abortion and mating system, coupled with a tentative evaluation of whether a significant component of detected seed abortion is selective.

Prediction 1. If low seed:ovule ratios result from selective abortion of less vigorous offspring, then outcrossing species should exhibit lower seed:ovule ratios than inbred species. A demonstrated positive correlation between seed:ovule ratios and selfing rate (fruit:flower ratio) would be consistent with the interpretation that seed:ovule ratios involve selective seed abortion.

Prediction 2. Seed abortion within a fruit should be nonrandom if the pattern of fertilizations within the ovary reflect a prezygotic sorting of genetic variants by microgametophytic selection.

We review variation in seed:ovule ratios and patterns of abortion within fruits as they relate to the predictions of the two hypotheses.

III. Seed:Ovule and Fruit:Flower Ratios

Methods

Data from two sources are presented below. The first is the published information, and wherever such data are presented, reference is made to the appropriate paper. The second source is a new data set resulting from recent observations of *Desmodium incanum* and *Gliricidia sepium* (Papilionoideae), *Senna pallida* and *S. bicapsularis* (Caesalpinoideae), and *Enterolobium cyclocarpum, Mimosa pigra, M. pusilla,* and *Pithecellobium saman* (Mimosoideae). These species are distributed in the dry deciduous forests of Guanacaste Province, Costa Rica (see Bawa & Webb, 1984, for a site description).

Seed:ovule ratios were calculated by dividing the number of mature seeds per fruit (regardless of predation injury) by the number of ovules per fruit. For this purpose, 14 to 386 randomly sampled fruits from three to nine individuals per species were examined (Tables 1, 3, 4). Observations of seed state were made by dissecting opaque fruits or by examining translucent fruits in front of a strong light source, depending upon species. The position of each seed within the fruit was also recorded in order to test hypotheses about the causes of seed abortion.

Fruit:flower ratios were calculated by dividing the average number of hermaphroditic flowers per inflorescence by the average number of fruits per infructescence, which was then multiplied by the fraction of inflorescences that became infructescences. Data were collected from four to twelve individuals per species, representing samples of from 96 to approximately 40,000 flowers per species (Tables 1, 3, 4).

Mating system, as defined in this paper, refers to the degree of inbreeding exhibited by the progeny arrays of individuals resulting from selfing or consanguineous mating (Clegg, 1980). The mating system was inferred from the breeding system, assuming that self-fertile species are more inbred than self-sterile species. Controlled pollinations were performed to test whether species are

TABLE 1. Seed:ovule and fruit:flower ratios in Papilionoideae.

Species	S:O Ratio[1]	Fr:Fl Ratio[2]	Mating System[3]
Desmodium incanum	0.81 ± 0.05 (7/64)	0.904 ± 0.03 (7/96)	I[6]
Gliricidia sepium	0.45 ± 0.05 (6/122)	0.0701 ± 0.006 (5/1582)	O[6]
Dalbergia retusa	0.239 ± 0.028 (3/240)[5]	0.081 ± 0.015 (5/560)[5]	O[4]
Myrospermum frutescens	0.196 ± 0.021 (4/348)[5]	0.031 ± 0.003 (4/3022)[5]	O[5]
Pterocarpus rohrii	0.160 ± 0.019 (5/386)[5]	0.071 ± 0.006 (5/1770)[5]	O[4]

[1,2]Mean and standard error, with parentheses containing: [1]number of sampled individuals/fruits or [2]number of sampled individuals/flowers.

[3]I = inbreeding, O = outcrossing.

[4]Data from Bawa, 1974.

[5]Data from Bawa & Webb, 1984.

[6]Data from Bawa, unpublished.

self-fertile or self-sterile. The sample sizes for such pollinations are given in Bawa (1974) and Bawa & Webb (1984). In fact, information about self-fertility for species reported here largely comes from our previous work, except for *Senna pallida, Desmodium incanum,* and *Gliricidia sepium,* which were recently investigated and cited as unpublished observations.

Apart from self-fertility, habit and density can also influence the degree of inbreeding. For example, we assume that gene dispersal via pollen or seed is more localized in herbs that grow in dense patches than in widely dispersed trees. A positive association between habit and outcrossing (Stebbins, 1958) and a negative association between density and gene dispersal (Levin & Kerster, 1974) are well documented.

PATTERNS

Papilionoideae

Table 1 shows seed:ovule ratios as well as fruit:flower ratios for five indigenous Guanacaste species. *Desmodium incanum* is an herb and self-fertile. Self-pollinated species generally have a higher fruit:flower ratio (Bawa, 1974; Sutherland & Delph, 1984). The fruit:flower ratio in *Desmodium incanum* is 0.86, as compared to 0.02–0.08 in the other four species, all of which are small trees and self-incompatible.

The seed:ovule ratio is highest in *Desmodium incanum* and lowest in *Pterocarpus rohrii.* In *Dalbergia retusa, Myrospermum frutescens,* and *Pterocarpus rohrii,* the average number of ovules per ovary is 4.78 ± 0.04, 5.14 ± 0.07, and 6.42 ± 0.04 respectively (Bawa & Webb, 1984), but the fruits are largely single-seeded and wind-dispersed. The part of the pod with aborted ovules and seeds is flattened to aid in dispersal. These three species with somewhat similar abortion rates have a lower seed:ovule ratio than *Gliricidia sepium,* whose seeds are dispersed by explosive longitudinal splitting of the fruit.

Overall, seed:ovule ratios closely approach unity in the self-fertilizing *Desmodium incanum* and are less than 0.5 in the self-incompatible species. This is in accord with the predictions of both Wiens (1984) and Bawa & Webb (1984), which implicated selective screening of genetic load and paternal combining ability as major factors in the abortion of seeds. Note also that variation in fruit:flower ratio parallels variation in seed:ovule ratio.

A relationship between high seed:ovule ratios and the breeding system has also been reported among *Lotus* congeners (Table 2). The ratios range from 0.24 to 0.54 in self-sterile species and from 0.87 to 1.0 in self-fertile species, except for *L. tetragonolobus,* which is self-fertile but exhibits the lowest seed:ovule ratio reported in the genus.

In the genus *Astragalus,* self-compatible *Astragalus asterhouti* has a higher seed:ovule ratio than the self-incompatible congeneric species (Karron, 1985).

TABLE 2. Seed:ovule ratio and mating systems in *Lotus* spp. (From Bubar, 1958).

Species	Seed:Ovule Ratio	Mating System
L. corniculatus	0.37	self-sterile
L. tenuis	0.50	self-sterile
L. uliginosus	0.54	self-sterile
L. weilleri	0.44	self-sterile
L. suaveolens	0.48	self-sterile
L. jacobaeus	0.24	self-sterile
L. corniculatus var. *japonicus*	0.95	self-fertile
L. angustissimus	1.00	self-fertile
L. hispidus	0.98	self-fertile
L. palustris	0.87	self-fertile
L. filicaulis	1.00	self-fertile
L. parviflorus	0.80	self-fertile
L. divaricatus	0.82	self-fertile
L. perigrinis	1.00	self-fertile
L. tetragonolobus	0.17	self-fertile

Variation in seed:ovule ratios as a function of the amount of outcrossing has also been reported within a species. In *Cajanus cajan*, the frequency of ovule abortion is negatively correlated with the amount of selfing (Cumaraswamy & Bawa, in prep.). Selfing propensity was measured in different accessions as the reported proportion of flowers that set fruit without manipulation in a greenhouse.

In summary, comparisons of conspecific populations, congeneric species, and higher taxa within the family clearly demonstrate that selfers exhibit higher seed:ovule ratios than outcrossers. Thus, these data are consistent with the first prediction.

Caesalpinioideae

The variation among caesalpinioid species for seed:ovule ratios and fruit:flower ratios is documented in Table 3.

First, there are no differences between the seed:ovule ratios of *Senna pallida*, a shrub, and *S. bicapsularis*, a tree, although we have some evidence that they differ in outcrossing rates. Our results from controlled pollinations show that *S. pallida* is self-compatible. The results for *S. bicapsularis* are ambiguous: the vast majority of both self- and cross-pollinated flowers failed to set fruit, but in some instances fruit from cross-pollinated flowers were retained longer and developed faster than the fruits from self-pollinated flowers. It may be significant that fruit development was meagre and fruit set zero from concurrent natural pollinations in nine of the ten individuals that were also artificially pollinated, although seven of those nine individuals did set abundant fruit later in the same season. The fruit:flower ratio for *S. bicapsularis* (0.09 ± 0.01) is much lower than that for *S. pallida* (0.60 ± 0.05). Fruit:flower ratios seem to be highly correlated with the degree of outcrossing in many species; self-incompatible species generally have much lower fruit:flower ratios than self-compatible species (Bawa, 1974; Bawa & Webb, 1984; Sutherland & Delph, 1984; Koptur, 1984). Based on very low fruit:flower ratios and the limited evidence from controlled pollinations, we presume that *S. bicapsularis* is largely self-incompatible. Our observations of low fruit:flower ratios in *S. bicapsularis* extend over ten flowering seasons of the same individuals.

Although *Senna pallida* is self-compatible, it is predominantly outcrossed, but to a lesser extent than *S. bicapsularis*. The large flowers (> 3 cm across) of *S. pallida* are pollinated by medium-sized to large bees that forage over long distances (Frankie & Coville, 1979). However, in contrast to *S. bicapsularis*, *S. pallida* has a patchy distribution in open fields, often forming dense colonies

TABLE 3. Seed:ovule and fruit:flower ratios in Caesalpinioideae.

Species	S:O Ratio[1]	Fr:Fl Ratio[2]	Mating System[3]
Bauhinia ungulata	0.59 ± 0.04 (5/189)	0.102 ± 0.006 (5/2180)	O[4]
Caesalpinia eriostachys	0.467 ± 0.027 (5/342)	0.010 ± 0.001 (12/11,205)	O[4]
Senna pallida	0.51 ± 0.05 (9/85)	0.60 ± 0.03 (8/266)	I[5]
Senna bicapsularis	0.51 ± 0.13 (5/14)	<0.091 ± 0.010 (5/2633)[6]	O?[5]

[1,2]Mean and standard error, with parentheses containing: [1]number of sampled individuals/fruits or [2]number of sampled individuals/flowers.

[3]I = inbreeding, O = outcrossing.

[4]Data from Bawa, 1974.

[5]Data from Bawa, unpubl.

[6]Fruit:flower ratios based on counts of immature fruits.

consisting of tens of individuals. The trees of *S. bicapsularis* are scattered in the forest, and mates are presumably recruited from a much wider area than is the case for *S. pallida*. The lower fruit:flower ratio in *S. bicapsularis*, which results from greater flower and young fruit abortion, may reflect the cost of increased outcrossing with the excess of flowers serving both male function (pollinator attraction) and/or selective screening of young fruits on the basis of paternal quality.

Second, among the outcrossing species, caesalpinioid seed:ovule ratios are somewhat higher than those for papilionoid legumes. This is due in part to differences in fruit morphology. Papilionoid *Myrospermum frutescens, Pterocarpus rohrii*, and *Dalbergia retusa* are predominantly single-seeded with seed:ovule ratios of 0.02, 0.16, and 0.24, respectively. Among caesalpinioid legumes, all species have fruits with more than 15 seeds and seed:ovule ratios ranging from 0.46 to 0.59. There are at least two possible explanations for why the seed:ovule ratios of multiseeded fruits should be higher than those of single-seeded fruits. Kress (1981) has proposed that an increase in the number of developing seeds per fruit may be correlated with the evolution of larger pollen loads arriving at the stigma to increase the genetic relatedness of embryos within fruits. Consequently, we may expect a positive correlation between seed:ovule ratios and number of seeds per fruit. Alternatively, high seed:ovule ratios may merely reflect a disproportionate abortion of fruits with fewer maturing seeds, as appears to be the case for species with multiseeded fruits (Lee & Bazzaz, 1982a, b; Bawa & Webb, 1984).

Mimosoideae

Seed:ovule ratios in Mimosoideae range from 0.66 to 1.0 (Table 4). The highest seed:ovule ratios in our sample were found in the two species of *Mimosa*. We believe that both species probably exhibit appreciable levels of inbreeding. *Mimosa pigra* is a large self-compatible shrub that grows gregariously along roadsides and especially streambanks. *Mimosa pusilla* is a small herb that forms dense colonies in old fields; its genetic neighborhood may be even more restricted than that of *M. pigra*. Although outcrossing may occur, mate recruitment takes place at a local level, certainly more locally than the large outcrossed trees of *Enterolobium cyclocarpum* and *Pithecellobium saman*.

Although high seed:ovule ratios indicate that selective screening through seed abortion is likely to be modest or absent, gametophytic selection is still possible. For example, the number of pollen grains per polyad in *Acacia collinsii, Enterolobium cyclocarpum*, and *Pithecellobium saman* exceeds the number of ovules per ovary (Table 5). We have also observed multiple polyad pollinations in both *P. saman* and *E. cyclocarpum*, as have Knox and his associates for *Acacia spp.* in Australia (Knox, pers. comm.), although the frequency of multiple polyad pollinations in unknown for any of the aforementioned species. The number of pollen grains per polyad exactly corresponds with the number of ovules per ovary only in the presumably highly inbred *Mimosa pusilla*. The situation is

TABLE 4. Seed:ovule and fruit:flower ratios in Mimosoideae.

Species	S:O Ratio[1]	Fr:Fl Ratio[2]	Mating System[3]
Acacia collinsii	0.66 ± 0.05 (7/173)	0.024 ± 0.006 (4/ca.24,000)	O[5]
Enterolobium cyclocarpum	0.85 ± 0.04 (8/81)	<0.0015 ± 0.0003 (4/ca.40,000)[4]	O[6]
Pithecellobium saman	0.78 ± 0.05 (7/68)	<0.0011 ± 0.0003 (4/ca.32,000)[4]	O[6]
Mimosa pigra	0.92 ± 0.03 (8/67)	0.052 ± 0.009 (8/ca.750)	I[7]
Mimosa pusilla	1.0 ± 0.0 (5/19)	0.25 ± 0.05 (6/ca.1,130)	I?

[1,2]Mean and standard error, with parentheses containing: [1]number of sampled individuals/fruits of [2]number of sampled individuals/flowers (flower number estimated as mean flower number per inflorescence times the number of infructescences observed).

[3]I = inbreeding, O = outcrossing.

[4]Fruit:flower ratios based on counts of immature fruits.

[5]Data from Janzen, 1974.

[6]Data from Bawa, 1974.

[7]Data from Bawa, unpublished.

TABLE 5. Relationship among habit, pollen units, ovule number, and pollination mechanism in Mimosoideae.

Species	Habit	Pollen Unit	Ovule Number	Pollination Mechanism
Mimosa pusilla	herb	4-grain tetrad	4	small bees
Mimosa pigra	shrub	4-grain tetrad	24	small or medium-sized bees
Acacia collinsii	small tree	16-grain polyad	8	small or medium-sized bees
Enterolobium cyclocarpum	large tree	32-grain polyad	16	small moths
Pithecellobium saman	large tree	32-grain polyad	20	large moths

quite different in *Mimosa pigra*, whose polyads are also four-grained but whose number of ovules per ovary is 24 (average number of seeds per fruit is 20). Multiple pollinations must be necessarily common in *M. pigra*, given that the seed:ovule ratio is so high (0.92 ± 0.03). It should also be noted that the high level of relatedness among pollen grains in single tetrads does not preclude gametophytic selection. Gametophytic selection can occur within as well as among meiotic arrays, and both kinds of selection can also occur following multiple polyad pollination.

Interestingly, the number of pollen grains per pollen unit and the pollen unit's size appear to co-vary with plant size and the degree to which pollination mode is specialized (Table 5). For example, the four-grained polyads of herbaceous *Mimosa pusilla* are smaller than the four-grained polyads of the shrubby *M. pigra*. Among trees, *Acacia collinsii* is a small tree with eight grained polyads, whereas *Pithecellobium saman* and *Enterolobium cyclocarpum* are each very large trees with 32-grained polyads. The flowers of *P. saman* are more than twice as large and are pollinated by larger moths than those of *E. cyclocarpum*. Similarly, there is a twofold difference in polyad size in favor of *P. saman*. We do not know if these relationships hold for other mimosoid legumes. The mimosoid legumes, given their diversity of habit, their variation in pollen grain number per polyad and their variability in pollination mechanisms, should provide an ideal material for comparative studies of the relationship between pollination mode and other life history traits.

In general, again among the outcrossing species, mimosoid ratios are higher than those of caesalpinioids and papilionoids, probably due to the uniqueness of pollen dispersal units within the subfamily. Pollen grains are assembled into polyads in the three outcrossing mimosoids sampled. Polyads increase the genetic relatedness of pollen grains arriving at the stigma such that selective screening of paternal genotypes is more likely among rather than within fruits. It is thus not surprising that fruit:flower ratios in mimosoid legumes examined are much lower than those in the other two legume subfamilies.

IV. Seed Abortion Within Fruits

The pattern of seed abortion is nonrandom in a number of species examined so far (Bawa & Webb, 1984; see review in Lee, 1988). In some species, seeds at the basal end (Bawa & Webb, 1984) and/or the stylar end (Horovitz et al., 1976; Bawa & Webb, 1984; Bawa, unpublished observation) of fruits exhibit higher abortion rates than those of other ovule positions. Bawa & Webb (1984) and Lee (1984) argued that nonrandom patterns of seed abortion might result from gametophytic selection and nonrandom patterns of fertilization within ovaries. According to this hypothesis, an important function of seed and fruit abortion in many species is selection for offspring quality, to compensate for uncertain paternal genetic quality and genetic load. This hypothesis predicts that the pattern of seed abortion following microgametophytic selection will depend on (1) the site of pollen tube entry into the style and (2) the relationship between gametophytic and embryonic phenotypic quality for genes subject to gametophytic selection (Figure 1).

Pollen Tube Entry Site

The gametophytic selection hypothesis predicts that ovules closest to the ovary's pollen tube entry site are likely to be fertilized by the sperm carried by the most vigorous pollen tubes. In contrast, the most distant ovules are likely to be fertilized by the least vigorous of the successful microgametophytes. In species whose pollen tubes enter the ovary's stylar (distal) end, microgametophytic selection will result in a gradient of embryos fertilized by decreasingly vigorous microgametophytic genotypes, from the style to the base of the fruit (Figure 1a). The gradient may be reversed in species whose pollen tubes enter the base (proximal end) of the ovary (Figure 1b; e.g., in *Lupinus nanus*, but not in *Medicago sativa*, see Lee, in press, for review). In species where pollen tube entry occurs at a median ovary level, a bimodal distribution is expected, resulting from two gradients of fertilizations by decreasingly vigorous microgametophytes, one from the entry site to the style and the other from the entry site to the basal end of the fruit (Figure 1c).

Correlations Between Microgametophytic and Sporophytic Vigor

Gametophytic selection is most likely to provide a useful prezygotic screen of subsequent embryo quality if gametophytically favored genotypes are also advantageous postzygotically in developing embryos. However, it is possible that some gametophytically favored factors will be disadvantageous in sporophytic development. The morphology and much of the physiology of the gametophytes and sporophytes are very different, and selection regimes may change in sign from one life cycle phase to the next, as Clegg et al. (1978) indicated. To the extent that gametophytically favored genotypes are disadvantageous to developing embryos, gametophytic selection may also increase the likelihood of abortion for those seeds fertilized by the most vigorous microgametophytes (i.e., closest to the site where pollen tubes enter the ovary; see Figure 1d–f). Negative correlations between gametophytic and embryonic vigor are not expected to be large, inasmuch as gametophytic selection experiments usually demonstrate a positive correlation between gametophytic selection intensities and progeny vigor (e.g., Mulcahy & Mulcahy, 1975; Mulcahy et al., 1975, 1978; Ottaviano et al., 1980).

However, nonrandom patterns of seed abortion can result from other causes, too (see Bawa & Webb, 1984, and Lee & Bazzaz, 1986, for reviews). For example, maternal resource availability might be greater at the base of the fruit, resulting in increasing seed abortion rates from the base to the stylar end of fruits, a prediction that differs from that of the gametophytic selection hypothesis except when the pollen tubes fertilize basal ovules first. Conversely, ovules at the base of the ovary

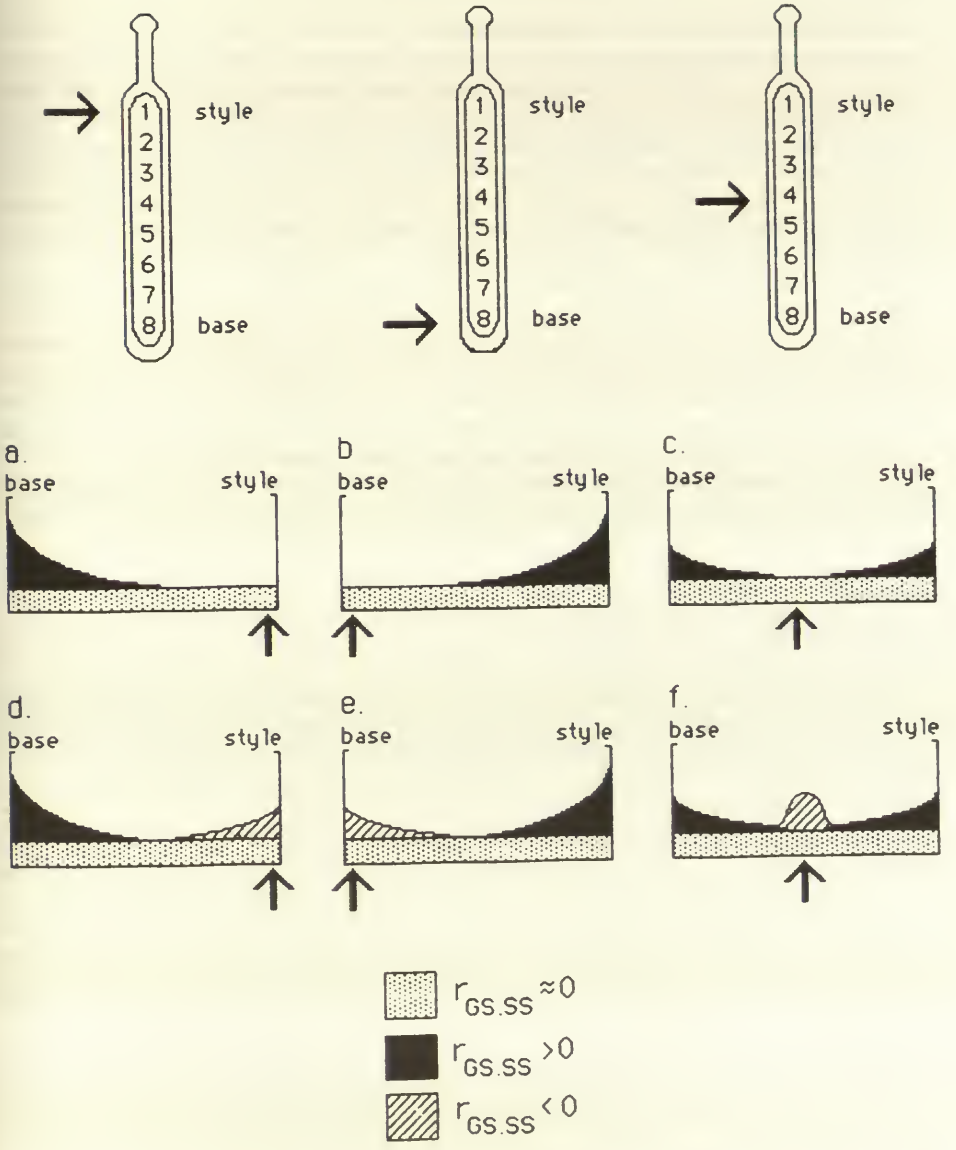

FIGURE 1. Expected patterns of seed abortion within fruits following microgametophytic selection. Arrows indicate the point at which pollen tubes enter the ovary. Fertilization sequence and vigor rank (1: most vigorous; 4–8: least vigorous) are indicated within the diagrammatic pistils. $r_{GS.SS}$ = correlation between microgametophytic and sporophytic selection for genes subject to microgametophytic selection (see text). —a–c. Only positive correlations considered. —d–f. Both positive and negative correlations considered.

might exhibit higher abortion rates if first-fertilized ovules at the stylar end of the ovary enjoy a temporal advantage in competition with them, although Lee & Bazzaz (1986) believed that a prominent pattern is not expected because temporal differences in fertilization time might be small. Seed abortion could also be under strict maternal control and nonselective, for example, in dispersal

of explosively dispersed seeds in *Cassia fasciculata* (Lee & Bazzaz, 1986). However, there is no reason to suppose that analogous processes are general phenomena of other legume seed dispersal syndromes. In our sample, 13 of 14 species exhibit nonrandom patterns of seed abortion within fruit, but only *Gliricidia sepium* and *Bauhinia ungulata* explosively disperse their seeds. Lastly, Watson & Casper (1984) suggested that patterns of ovule abortion within fruit are determined by vascular anatomy and translocation and represent a phylogenetic constraint, not a selection mechanism. If so, then patterns of seed abortion are not expected to correlate with mating system. Nor does this latter hypothesis explain the many species whose basal seed positions exhibit the highest abortion rates (Lee, 1988).

In the following, we will make the provisional assumption that pollen tube entry sites are situated at the stylar end of the ovaries. According to the gametophytic selection hypothesis, a positive correlation between seed abortion rate and more proximal seed position results from microgametophytic selection and selective seed abortion within fruits. We consider that the gametophytic selection hypothesis would be the most parsimonious explanation for the predicted seed abortion pattern should that pattern turn out to be prominent, taxonomically widespread, correlated with mating system, and independent of variation in seed dispersal syndrome.

In order to quantify seed abortion pattern within fruits, seed abortion rates per seed position were subjected to polynomial regression analysis on seed position (numbered from fruit base to style).

PATTERNS

Papilionoideae

Among the five papilionoid species studied, three exhibit predominantly single-seeded fruits, flattened to aid in wind dispersal *(Dalbergia retusa, Myrospermum frutescens,* and *Pterocarpus rohrii).* Even in these species, where the fruit morphology imposed by the dispersal mechanism might presumably favor median seed maturation, seed abortion rates are higher in proximal seed positions (Bawa & Webb, 1984).

The two remaining papilionoid species exhibit higher seed:ovule ratios. Again, the pattern of seed abortions is nonrandom (Table 6, Fig. 2), but early and late seed abortion rates differ in degree and overall pattern. In *Gliricidia sepium* fruits, 46% of seeds mature, 46% abort very early and 8% abort at a late stage of development. Among early abortions, both distal (Table 6a, positive quadratic component, $P = 0.0002$) and proximal (Table 6a, negative linear component, $P = 0.0004$) seed positions exhibit higher early abortion rates than median positions, especially the most proximal positions (Fig. 2). Late abortion rates are constant in the basal two-thirds of fruits but show an increase in the stylar end (Table 6b). The increasing late abortion rate in the stylar end of *G. sepium* fruits parallels the early abortion rates at the same seed positions (correlation between early and late abortion rates in the distal 40% of the fruit: $r = 0.995$, 2df, $P = 0.005$).

Bawa & Webb (1984) suggested that enhanced seed abortion rates in proximal (basal) regions of the fruit may be due to gametophytic selection or to temporal disadvantage suffered by later fertilized ovules in competition with earlier fertilized distal ovules. However, the second explanation is not consistent with the observation that distal abortion rates are also higher than median rates, unless the greater frequency of distal abortions simply reflects decreased availability of maternal resources at that end of the fruit. The colinearity of early and late abortion rates at proximal seed positions indicates that the two curves may reflect the same process, and it seems unlikely that a maternal resource gradient within fruits would be identical in effect on both very early and late abortions. However, enhanced distal seed abortion rates are not inconsistent with the gametophytic selection model. In contrast to enhanced proximal abortion rates, which would represent the elimination of genotypes that were relatively disadvantageous to both microgametophytes and embryos, enhanced distal abortion rates would be expected for genetic variants that are advantageous during pollen tube growth but disadvantageous during embryogeny. If this interpretation is correct, then

TABLE 6. Seed abortion patterns within fruits: polynomial regressions of seed abortion rate per seed position on seed position. a–g = prediction models. P = seed position within fruit (numbered from fruit base to stylar end); R^2 = coefficient of determination (fraction of variance explained by model).

Species	Model	Source	Degrees of freedom	Mean squares	F-ratio	P
Gliricidia sepium (Fig. 2):						
	a. Early abortion rate = $0.713 - 0.097P + 0.0073\,P^2$; $R^2 = 0.906$					
		model	2,7	0.02524	44.573	0.0001
		linear	1,7	0.02237	39.503	0.0004
		quadratic	1,7	0.02812	49.644	0.0002
	b. Late abortion rate = $0.095 - 0.0026P^2 + 0.00031P^3$; $R^2 = 0.710$					
		model	2,7	0.0054	11.882	0.006
		quadratic	1,7	0.0028	12.314	0.009
		cubic	1,7	0.0026	11.451	0.011
Bauhinia ungulata (Fig. 4):						
	c. Early abortion rate = $0.582 - 0.0571P + 0.00199P^2$; $R^2 = 0.818$					
		model	2,18	0.1124	45.966	$< 10^{-6}$
		linear	1,18	0.1357	55.580	$< 10^{-4}$
		quadratic	1,18	0.0891	36.451	$< 10^{-4}$
Senna pallida (Fig. 5):						
	d. Early abortion rate = $0.593 - 0.0580P + 0.0028P^2 - 3.8 \times 10^{-5}P^3$; $R^2 = 0.681$					
		model	3,31	0.0669	25.178	$< 10^{-6}$
		linear	1,31	0.0241	9.084	0.0051
		quadratic	1,31	0.1434	53.974	$< 10^{-4}$
		cubic	1,31	0.0331	12.476	0.0013
Mimosa pigra (Fig. 7):						
	e. Total abortion rate = $0.7348 - 0.3881P + 0.0635P^2 - 0.0032P^3$; $R^2 = 0.926$					
		model	3,6	0.0517	38.281	0.0003
		linear	1,6	0.0648	47.936	0.0004
		quadratic	1,6	0.0586	43.355	0.0006
		cubic	1,6	0.0318	23.550	0.0028
Enterolobium cyclocarpum (Fig. 8):						
	f. Total abortion rate = $0.2940 - 0.0229P + 0.00082P^2$; $R^2 = 0.369$					
		model	2,17	0.0164	6.435	0.008
		linear	1,17	0.0210	8.225	0.011
		quadratic	1,17	0.0118	4.644	0.046
Pithecellobium saman (Fig. 9):						
	g. Total abortion rate = $0.6779 - 0.0820P + 0.0032P^2$; $R^2 = 0.900$					
		model	2,23	0.3447	112.891	$< 10^{-6}$
		linear	1,23	0.0237	7.7481	0.011
		quadratic	1,23	0.6658	218.034	$< 10^{-4}$

gametophytically selected genes disadvantageous to both pollen and embryos might be eliminated very early in embryogeny, whereas gametophytically favored genes that impair embryo performance are screened during both early and late embryogeny. This interpretation makes developmental sense in that the epigenetic background of embryos almost certainly becomes progressively less gametophyte-like during embryogeny.

As predicted, autogamous *Desmodium incanum* exhibits a higher seed:ovule ratio and less variability in seed abortion rates within fruits than outcrossed *Gliricidia*. Notwithstanding these differences, patterns of seed abortion within fruit (Fig. 3) are still nonrandom. Early abortion rates exhibit a linear decrease within fruits from the fruit base to the stylar end ($P = 0.033$; 1,4 df), indicating that perhaps 70% ($r^2 = 0.717$) of early seed abortion rate variability within fruits may involve postzygotic screening of genetic variants that have been sorted prezygotically by gametophytic selection.

FIGURE 2. Seed abortion patterns within fruits of *Gliricidia sepium*. Lighter line = early abortion rates; darker line = late abortion rates.

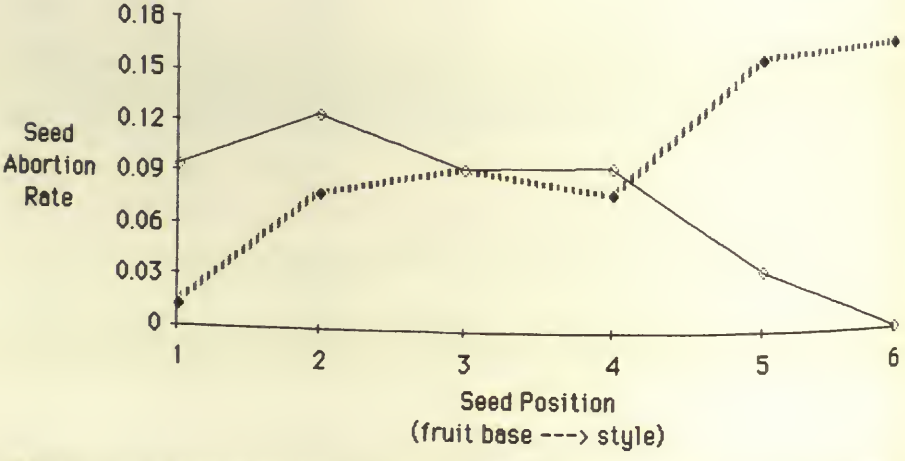

FIGURE 3. Seed abortion patterns within fruits of *Desmodium incanum*. Solid line = early abortion rates; broken line = late abortion rates.

Caesalpinioideae

All four caesalpinioids exhibit seed:ovule ratios similar to *Gliricidia sepium*, whose habit, fruit morphology, and probably breeding system is somewhat similar to theirs. However, patterns of seed abortion are more complex in the caesalpinioid species.

In *Caesalpinia eriostachys*, seed abortion rates decrease in a linear fashion from proximal to distal ovule positions (Bawa & Webb, 1984). In *Bauhinia ungulata* (Table 6c, Fig. 4) and *Senna pallida* (Table 6d, Fig. 5), early seed abortion rates decrease dramatically from proximal to median positions (Table 6c,d: both with negative linear components, both $P < 0.01$) and then

FIGURE 4. Seed abortion patterns within fruits of *Bauhinia ungulata*. Lighter line = early abortion rates; darker line = late abortion rates. (Redrawn from Webb & Bawa, 1985.)

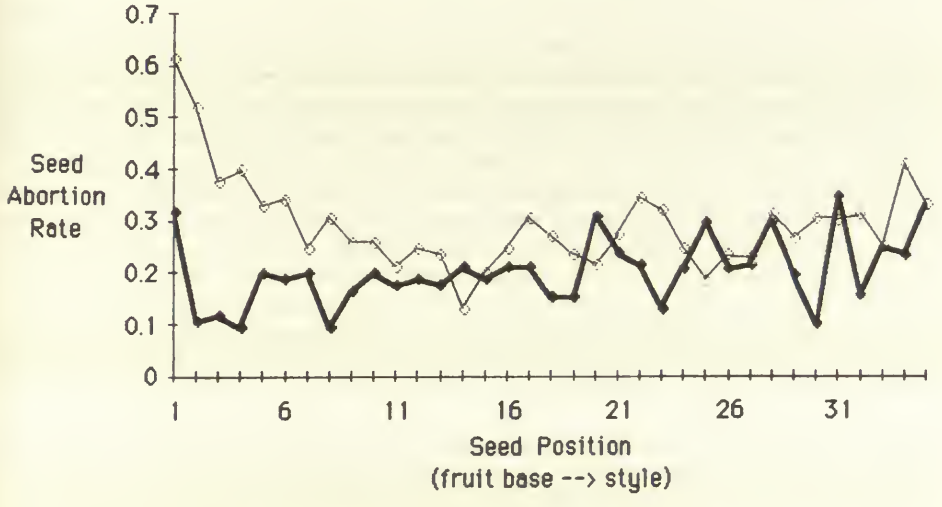

FIGURE 5. Seed abortion patterns within fruits of *Senna pallida*. Lighter line = early abortion rates; darker line = late abortion rates.

increase again from median to distal positions (Table 6c,d: both with positive quadratic components, both $P < 0.01$). These seed abortion patterns are consistent with the interpretation that gametophytic selection is influencing the pattern of embryo vigor and seed abortion, which would also indicate that seed abortion is selective. Late seed abortion rates show a different pattern within fruits in both species, a linear increase from proximal to distal seed positions (*B. ungulata:* $r = 0.438$, 19 df, $P = 0.027$; *S. pallida:* $r = 0.383$, 31 df, $P = 0.013$). This pattern of late seed abortions could result from a maternal resource gradient within the fruits and/or from selective abortion of gametophytically selected factors that were disadvantageous during later stages of embryogeny.

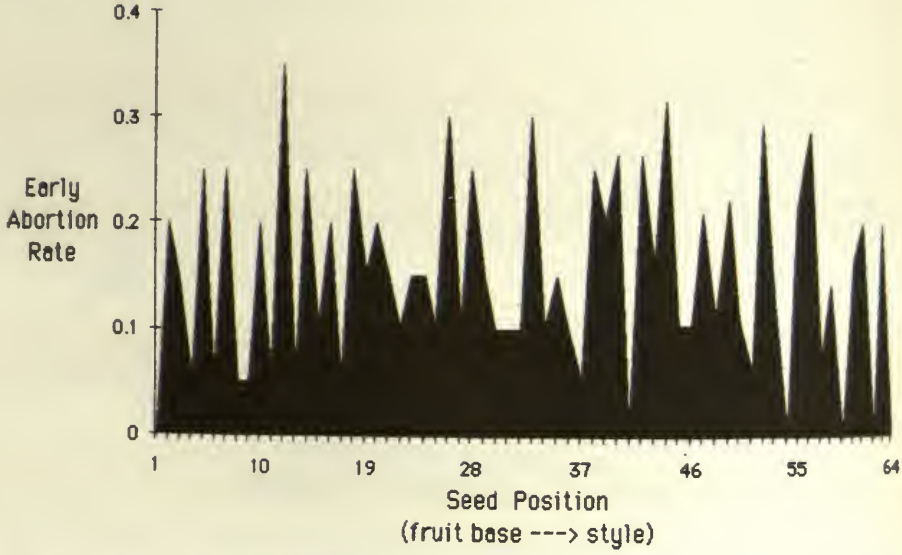

FIGURE 6. Pattern of early seed abortion within fruits of *Senna bicapsularis*.

When seed abortion rates are plotted against seed position within fruits, a very pronounced difference is apparent between the curves of *Bauhinia ungulata* and *Senna pallida* (Figs. 4, 5), compared with the very smooth curves of the papilionoid species (Figs. 2, 3) and *Caesalpinia eriostachys* (Bawa & Webb, 1984). *Bauhinia ungulata* and especially *S. pallida* exhibit an alteration of very high and very low abortion probabilities over very small fruit regions corresponding to sequences of only a few seeds in length. This pattern may indicate that abortion probabilities differ for the two fruit valves, with no selection implicated. Alternatively, local variation in abortion rates within fruits might result from greater competition among neighboring seeds. At least two factors could cause greater interaction among seeds within fruits in these species: (1) much higher ovule numbers per ovary might make individual ovules more expendable (20–35 vs. 6–10 ovules/ovary), and (2) shorter style lengths and correspondingly lessened intensities of gametophytic selection might increase the benefits of seed competition within fruits. Consistent with the selection hypothesis is the observation that the amplitude of neighboring high and low late abortion proababilities in *S. pallida* is much higher for distal than proximal halves of mature fruits (Figure 5), which might result from greater competition among embryos fertilized by the most competitive microgametophytes or from greater interaction among neighboring seeds in regions of fruits where maternal resources are most limiting.

Lastly, the pattern of local variation in seed abortion rates in *Senna bicapsularis* fruits (Fig. 6) is even greater than that in *Bauhinia ungulata* or *Senna pallida*. Again, this may be due simply to unequal abortion rates in the two fruit valves. If such pronounced local variation in seed abortion rates within fruits is due to competitive interactions or interference between developing seeds, then seed abortion rates in many-seeded fruits might be influenced more strongly by interactions among neighboring seeds than by gametophytic selection in *S. bicapsularis*. It would not be surprising that gametophytic selection is relatively less important in *S. bicapsularis* than in its congener. First, the flower abortion and fruit selection might play a more significant progeny screening role in *S. bicapsularis* than in *S. pallida*. Second, even though preliminary data show that the overall seed abortion rates of the two species are similar, early seed abortion rates are approximately twice as high in *S. pallida* as they are in *S. bicapsularis*.

In both papilionoids and caesalpinioids, early seed abortion rates show the clearest evidence of having been influenced by strong gametophytic selection.

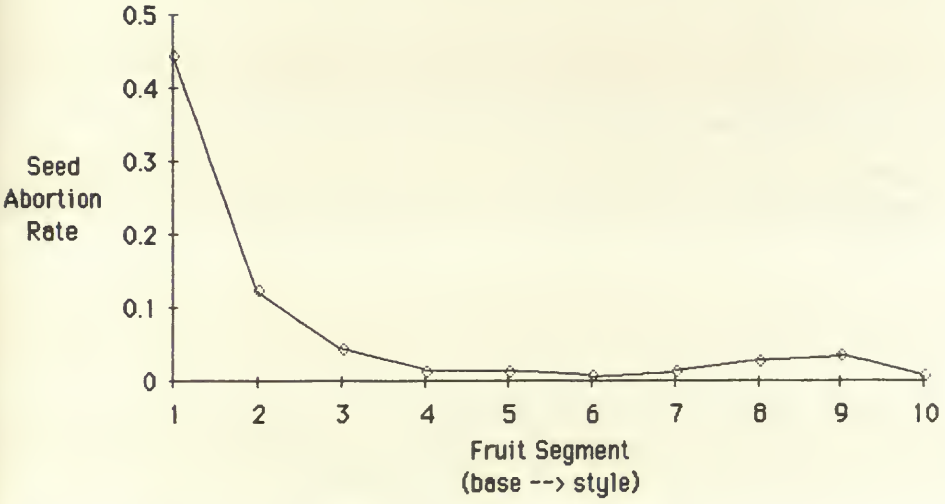

FIGURE 7. Total seed abortion pattern within fruits of *Mimosa pigra*.

Mimosoideae

Both *Mimosa pigra* and *Enterolobium cyclocarpum* exhibit higher seed abortion rates in basal than stylar fruit regions, which is consistent with the interpretation that some degree of seed abortion is selective. The pattern is especially pronounced for *M. pigra*, in which seed abortions are concentrated at the proximal end of fruits (Table 6e, Fig. 7). In contrast, seed abortion rates are appreciable throughout the fruits of *E. cyclocarpum*, with proximal rates only marginally higher (Table 6f, Fig. 8). One factor that might be responsible for these differences in seed abortion pattern is that *M. pigra* might be more highly inbred than *E. cyclocarpum*, as previously suggested, resulting in more predictable paternal genetic quality.

Seed abortion patterns are nonrandom within *Pithecellobium saman* fruits as well (Table 6g), but the pattern is reversed compared with the other sampled species exhibiting a nonrandom pattern. Both the basal and the stylar ends of the fruits exhibit higher seed abortion rates than the central region of the fruit, but the stylar abortion rates are the highest (Fig. 9a). It is possible that maternal resources are exceptionally limiting for the distal (stylar) seeds in *P. saman* fruits, compared with fruits of other species. This pattern is also predicted by the gametophytic selection hypothesis under two related circumstances: (1) pollen tube entry is near the center of the ovary but closer to the base than the style, and the influence of microgametophytic selection on seed abortion is predominantly restricted to positive correlations between microgametophytic and sporophytic vigor (Fig. 9b, similar to Fig. 1c); or (2) pollen tubes enter the base of the ovary, and some significant minority of gametophytically favored genes decreases sporophytic vigor and increases the seed abortion rate correspondingly (Figs. 1e, 9c).

Overall, the surveyed mimosoid seed abortion rate curves (Figs. 7, 9, and to a lesser extent 8) are smoother and show a more consistent relationship to seed position than the caesalpinioid curves, which are more ragged (Figs. 4, 5, 6). A possible explanation is that seeds within fruits of mimosoids have a higher genetic relatedness due to pollination by polyads than do the seeds of ceasalpinioids, and thus sibling competition is reduced (see Kress, 1981). Of course, the papilionoid abortion rate curves (Figs. 2, 3) are the smoothest of all three subfamilies, but the species we examined have small seed number per fruit as compared with the species of the other two subfamilies.

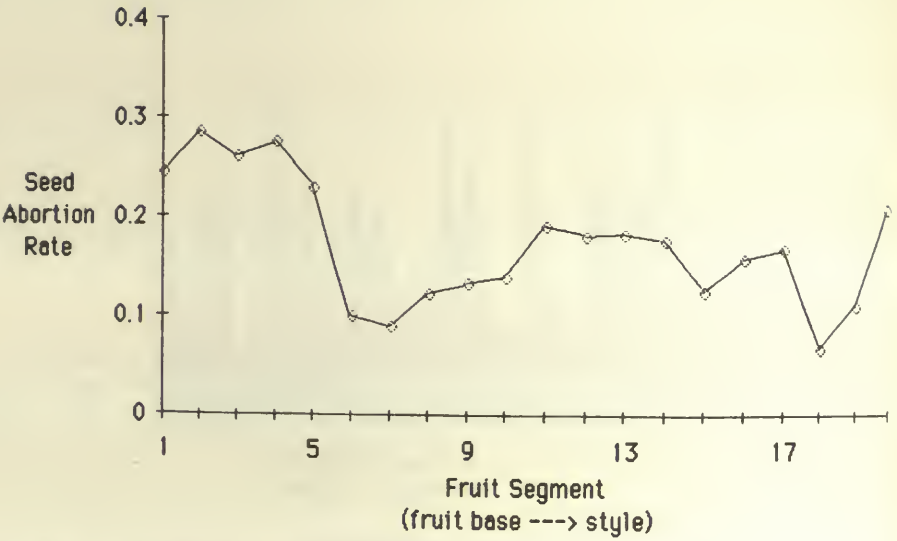

FIGURE 8. Total seed abortion pattern within fruits of *Enterolobium cyclocarpum*.

V. DISCUSSION

All of the species' seed:ovule and fruit:flower ratios surveyed are less than one, except for the seed:ovule ratio of *Desmodium incanum*. The results are clearly in accord with the predictions. Before we offer further commentary, however, we should stress that failure to mature seed or to set fruit is not necessarily due to lack of pollination or compatible pollen, although the most parsimonious explanation for high seed and fruit set in selfing species is that pollination levels are very high under autogamy. But in outcrossing species, abortion of flowers, fruits, and seeds occurs even when flowers are adequately pollinated (see Bawa & Webb, 1984, for detailed discussion). Furthermore, within fruits, abortion of developing seeds can actually be observed in all the species reported herein. Nonetheless, we do believe that in outcrossing species abortion of some of the ovules (and flowers) must be due to lack of pollination. Moreover, in some legumes, embryo sacs in a certain, although an unknown fraction of ovules degenerate before pollination (Bubar, 1957). Ovules aborted due to lack of pollination and ovules with degenerated embryo sacs can be distinguished, and we believe such ovules accounted for a small fraction of the total abortions considered in this paper (Bawa & Buckley, unpublished observation).

Our data clearly show that outcrossers have lower seed:ovule ratios than selfers or inbreeders. Outcrossing populations are expected to carry a considerable amount of genetic load, and a certain fraction of abortions may be ascribed to this load (Wiens, 1984; Bawa & Webb, 1984). However, we also note that the frequency of abortion declines among outcrossing species as the uncertainty about the quality of pollen genotypes arriving at the stigma decreases, as for example in Mimosoideae. The mode of seed dispersal also seems to influence seed:ovule ratios. Apparently, we have barely begun to discern the factors influencing seed:ovule ratios in plants.

We have also shown that the abortion of seeds within fruits is nonrandom and have argued that, in most cases examined, microgametophytic selection plays a leading role in causing the nonrandomness. Meinke (1982) has presented a more cogent case for nonrandom abortion of seeds within a fruit resulting from prezygotic screening of recessive lethals or sublethals by gametophytic selection. However, Lee & Bazzaz (1986) have demonstrated legume seed abortion patterns similar to those described herein that cannot be accounted for by genetic load for lethals or sublethals.

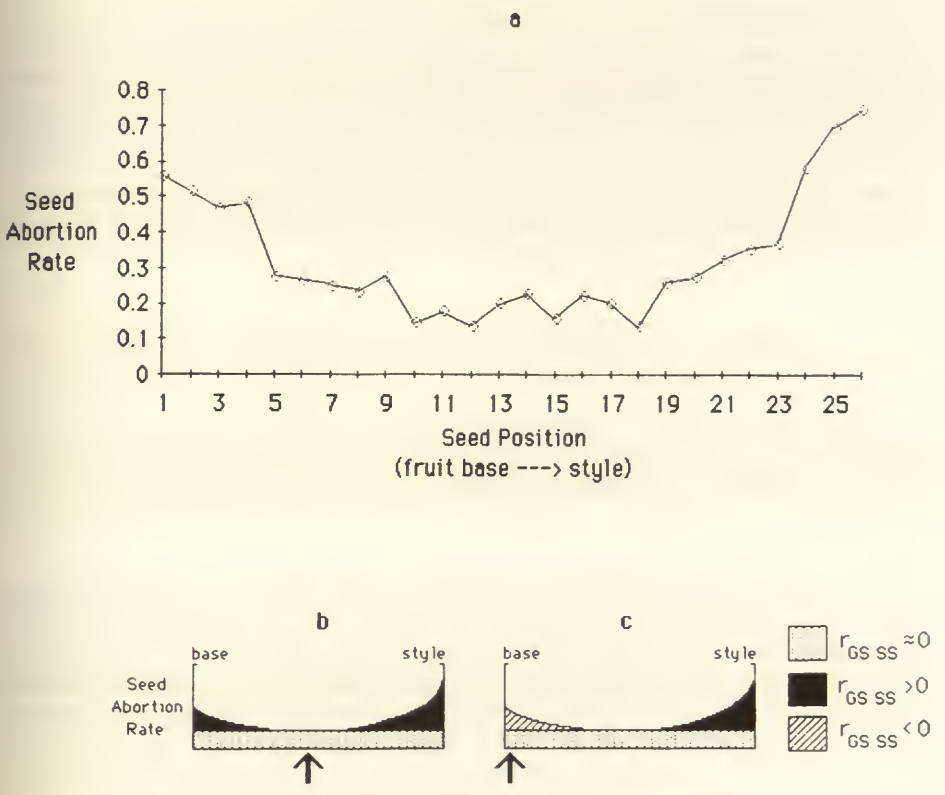

FIGURE 9. —a. Total seed abortion pattern within fruits of *Pithecellobium saman*. —b, c. Explanatory hypotheses implicating gametophytic selection (see text). Arrows indicate the point at which pollen tubes enter the ovary. $r_{GS.SS}$ = correlation between microgametophytic and sporophytic selection for genes subject to microgametophytic selection.

Although we have not fully addressed apparent differences between early and late seed abortions, seed mortality is a continuous process, with different selection regimes being relatively more or less important at different stages. For example, the influence of microgametophytic selection and the selective elimination of genetic load might be more important in early abortion, whereas competitive interactions and the degree of genetic relatedness among seeds might assume greater significance in later abortion. In subsequent papers, we expect to distinguish between early and late abortions and their underlying causes.

Our observations also confirm that if our goal is to determine how flowers are used as sites of selection to optimize the genetic quality of the offspring, then seed:ovule ratios must be examined in conjuction with fruit:flower ratios. This is clearly evident in the case of Mimosoideae. Seed:ovule ratios are uniformly high even in outcrossing species, but fruit:flower ratios are extremely low. Selection in mimosoid legumes is exercised at the level of flowers rather than at the level of ovules or seeds within the fruits. Polyads increase the genetic relatedness among pollen grains fertilizing the ovules within an ovary. Thus the almost full seed set in the very small fraction of flowers that are developed into fruits in Mimosoideae is not a mere coincidence. In Caesalpinioid legumes, the self-compatible *Senna pallida* and the presumably more widely outcrossed *S. bicapsularis* have similar seed:ovule ratios but widely disparate fruit:flower ratios. In *S. bicapsularis* there is more selection at the level of flowers than in *S. pallida*.

Finally and most important, by examining the abortion of reproductive structures (flowers, fruits, and seeds), we seek to understand when and where selection operates from the time the pollen grains land on the stigmas to the time the seeds are mature. The intensity of this selection and whether selection occurs primarily among flowers or developing seeds within a fruit determines the relative amount of resources allocated to the production of flowers and fruits and the evolution of such traits as inflorescences, pollen units, and ovule number (Bawa & Webb, 1984). Because of rich diversity in mating and pollination systems, legumes provide ideal material for comparative studies aimed at determining the relationship between gametophytic and embryo selection and the evolution of inflorescences, pollen units, and ovule number.

Acknowledgments

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Addendum

Since the symposium was held in late June 1986, several papers on the subject have been published. Three of those papers bear directly on the issues covered in this paper and are briefly considered below.

First, Wiens et al. (1987) further elaborated on ideas of genetic load, expressed earlier by Wiens (1984). Nevertheless, their predictions and conclusions remain discordant with those presented here.

Second, Wojciechowska (1986) presented additional data to show that in Papilionoideae there might be correlation between seed set and the breeding system.

Third, at the time this paper was written, we did not consider the role of parent–offspring conflict in seed abortion. This conflict also explains the currently observed patterns of abortion, especially in papilionoid legumes that have their seeds dispersed by wind (see Uma Shaanker et al., 1988). In models of parent–offspring conflict, seed abortion may result from selection enhancing the fitness of offspring, and, as also argued here, genetic relatedness of seeds within the ovary greatly influences the intensity of abortion (Uma Shaanker et al., 1988). Predictions of hypotheses based on the maternal regulation of offspring quality and the parent–offspring conflict are somewhat similar, especially those relating to the effect of mating system on seed abortion. Nevertheless, validity of the parent–offspring model may negate some of the conclusions reached in this study. Additional theoretical as well as empirical work is required to discriminate the predictions of the two hypotheses.

References

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85–92.

——— & C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. Amer J. Bot. 71: 736–751.

Bookman, S. S. 1984. Evidence for selective fruit production in *Asclepias*. Evolution 35: 72–86.

Bubar, J. S. 1958. An association between variability in ovule development within ovaries and self-incompatibility in *Lotus* (Leguminosae). Canad. J. Bot. 36: 65–72.

Buchholz, J. T. 1922. Developmental selection in vascular plants. Bot. Gaz. (Crawfordsville) 73: 249–286.

Clegg, M. T. 1980. Measuring plant mating systems. Bioscience 30: 814–818.

_____, A. L. Kahler & R. W. Allard. 1978. Estimation of life cycle components of selection in an experimental plant population. Genetics 89: 765–792.

Frankie, G. W. & R. Coville. 1979. An experimental study on the foraging behaviour of selected solitary bee species in the Costa Rica dry forest (Hymenoptera: Apoidea). J. Kansas Entomol. Soc. 52: 591–602.

Horovitz, A., L. Meiri & A. Beiles. 1976. Effects of ovule positions in fabaceous flowers on seed set and outcrossing rates. Bot. Gaz. (Crawfordsville) 137: 250–254.

Janzen, D. H. 1974. Swollen thorn *Acacias* of Central America. Smithsonian Contr. Bot. 13: 1–131.

_____. 1977. A note on optimal mate selection by plants. Amer. Naturalist 111: 365–371.

Karron, J. D. 1985. Pollinator visitation rates, self-compatibility and genetic structure in geographically widespread and restricted species of *Astragalus* (Fabaceae). Bull. Ecol. Soc. Amer. 66: 206.

Koptur, S. T. 1984. Outcrossing and pollinator limitation of fruit set: breeding systems of neotropical *Inga* trees (Fabaceae: Mimosoideae). Evolution 38: 1130–1143.

Kress, W. J. 1981. Sibling interaction and the evolution of pollen unit, ovule number and pollen vector in angiosperms. Syst. Bot. 6: 101–112.

Lee, T. D. 1984. Patterns of fruit maturation: A gametophyte competition hypothesis. Amer. Naturalist 123: 427–432.

_____. 1988. Patterns of fruit and seed production. Pp. 179–202 *in* J. Lovett-Doust & L. Lovett-Doust (editors), Reproductive Strategies of Plants. Oxford Univ. Press, Oxford.

_____ & F. A. Bazzaz. 1982a. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. Ecology 63: 1364–1373.

_____ & _____. 1982b. Regulation of fruit maturation pattern in annual legume, *Cassia fasciculata*. *Ecology* 63: 1374–1388.

_____ & _____. 1986. Maternal regulation of fecundity: non-random ovule abortion in *Cassia fasciculata*. Michx. Oecologia 68: 439–465.

Levin, D. A. & H. W. Kerster. 1974. Gene flow in seed plants. Evol. Biol. 7: 139–220.

Lloyd, D. G. 1979. Parental strategies of angiosperms. New Zealand J. Bot. 17: 595–606.

Meinke, D. W. 1982. Embryo-lethal mutants of *Arabidopsis thaliana:* Evidence for gametophytic expression of the mutant genes. Theor. Appl. Genet. 63: 381–386.

Mulcahy, D. L. 1979. The rise of angiosperms: a genecological factor. Science 206: 20–23.

_____ & G. B. Mulcahy. 1975. The influence of the gametophytic competition on sporophytic quality in *Diantus chinensis*. Theor. Appl. Genet. 46: 277–280.

_____, _____ & E. Ottaviano. 1975. The influence of gametophytic competition in *Petunia hybrida*. *In:* D. L. Mulcahy (editor), Gamete Competition In Plants and Animals. North-Holland, Amsterdam.

_____, _____ & _____. 1978. Further evidence that gametophytic selection modifies the genetic quality of the sporophyte. Soc. Bot. Fr., Actualites Botaniques 2: 57–60.

Nakamura, R. R. 1986. Maternal investment and fruit abortion in *Phaseolus vulgaris* L. Amer. J. Bot. 73: 1049–1057.

Ottaviano, E., M. Sari-Gorla & D. L. Mulcahy. 1980. Pollen tube growth rates in *Zea mays:* implications for genetic improvement of crops. Science 210: 437–438.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.

_____. 1958. Longevity, habitat, and release of genetic variability in the higher plants. Cold Spring Harbor Symp. Quant. Biol. 23: 365–378.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Rev. Ecol. Syst. 12: 253–279.

Sutherland, S. & L. Delph. 1984. On the importance of male fitness in plants: patterns of fruit set. Ecology 65: 1093–1104.

Temme, D. H. 1986. Seed size variability: a consequence of variable genetic quality among offspring? Evolution 40: 414–417.

Uma Shaanker, R., K. Ganeshaiah & K. S. Bawa. 1988. Parent-offspring conflict, sibling rivalry and brood size patterns in plants. Ann. Rev. Ecol. Syst. 19: 177–205.

Watson, M. A. & B. B. Casper. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Annual Rev. Ecol. Syst. 15: 233–258.

Webb, C. J. & K. S. Bawa. 1985. Patterns of fruit and seed production in *Bauhinia ungulata* (Leguminosae). Pl. Syst. Evol. 151: 55–65.

Westoby, M. & B. Rice. 1982. Evolution of the seed plants and inclusive fitness of plant tissues. Evolution 36: 713–724.

Wiens, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64: 47–53.

_____, C. L. Calvin, C. A. Wilson, C. I. Davern, D. Frank & S. R. Seavey. 1987. Reproductive success, spontaneous embryo abortion and genetic load in flowering plants. Oecologia 71: 501–509.

Wojciechowska, W. 1986. Wstepne rozpoznawanie sposobu zapylenia u roślin motylkowych. Roczniki Akademii Rolniczej w Poznaniu. CLXVIII: 157–174.

Zamir, D. 1983. Pollen gene expression and selection. *In* S. D. Tanksley & T. J. Orton (editors), Isozymes in Plant Genetics and Breeding, Part A. Elsevier, Amsterdam.

THE FLORAL ECOLOGY OF AUSTRALIAN *ACACIA*

Peter Bernhardt[1]

Abstract

Although the floral phenology of Australian *Acacia* species is rather variable, the majority of southeastern species flower from August through October. The primary attractants include yellow, mass-flowering inflorescences and a range of scents. The polyad appears to be the major edible reward, although most pollinators will consume extrafloral nectar when it is available. Pollinators span three insect orders and three families of perching birds. Native Apoidea, of the short-tongue families Halictidae and Colletidae, probably effect the majority of cross-pollinations. The bees that pollinate *Acacia* are polylectic, and foraging bouts on inflorescences are usually punctuated by visits to co-blooming, nectariferous flowers, especially myrtaceous shrubs and trees. Most pollinations occur by automimicry when polyad laden insects attempt to forage on female phase (protogynous) inflorescences. Pollen collection by birds visiting *Acacia terminalis* is passive as birds brush against inflorescences while attempting to collect nectar from extrafloral glands on the leaf petioles. Inflorescence presentation appears sufficiently plastic to accommodate fluctuations in the density and diversity of pollinators over the distribution of an *Acacia* species. However, major shifts in the pollinator spectrum may lead to incipient speciation as reflected in divergent morphological/physiological characters of allopatric populations.

> "It is the work of the bees of Bayamee to make Yarrageh the east wind blow rain down the mountain, that the trees may blossom and the earth bees make honey."
> —Aboriginal legend collected by K. L. Parker.

I. Introduction

Australia is the country of *Acacia*, with over 700 *Acacia* species (Pedley, 1978–1979). As the continent is explored, that number may be found to approximate 1,200.

Predictably, advances in the study of anthecology lag far behind those in alpha taxonomy. Excluding some research in the Neotropics (Janzen, 1974; Zapata & Arroyo, 1978), references to the floral ecology of *Acacia* remain mostly anecdotal and fragmentary; and the number of *Acacia* species that have been rigorously researched is small compared to the size of the genus in Australia. Considering the continual advance of deserts and the economic importance of *Acacia* in the wood pulp, tanning, perfume, and horticultural industries, this level of ignorance is unfortunate. The evidence presented below should be interpreted as a stimulus to future fieldwork on the evolution of pollination systems within this genus.

II. Flowering Behavior

Floral phenology

Acacia flowers throughout the year in Australia. Species distributed through the arid center of the continent (e.g., *A. aneura*) tend to have irregular flowering seasons and are most likely to blossom after heavy rains (Costermans, 1983). Coastal populations are more likely to have proscribed flowering seasons varying according to latitude and climate.

A large proportion of Australian *Acacia* species flower from late winter until mid spring. Known as "wattle season," this is a period when floral phenologies show intense, interspecific overlap.

[1]Department of Biology, Saint Louis University, St. Louis, Missouri 63103, U.S.A.

C. H. Stirton & J. L. Zarucchi (eds.)—Advances in Legume Biology
Monogr. Syst. Bot. Missouri Bot. Gard. 29:263–281. 1989.

TABLE 1. Flowering and protogynous phase of *Acacia* species over their respective flowering seasons.

Flowering Phase (as a ratio)	Mean of Each Species[a]			
	MI	PY	RR	TE
Ratio of inflorescences in flower over the total number of inflorescences on a raceme	0.67	0.97	0.86	0.43
Ratio of inflorescences in the female phase over the number of inflorescences in flower on a raceme	0.14	0.21	0.16	0.21
Ratio of racemes bearing male phase inflorescences only	0.64	0.54	0.61	0.43
Ratio of racemes bearing inflorescences in the female phase only	0.05	0.14	0.05	0.04

[a]MI = *Acacia mitchelii* (number of racemes counted twice per week over the flowering season = 90); PY = *A. pycnantha* (n = 109); RR = *A. retinodes* var. *retinodes* (n = 80); TE = *A. terminalis* (n combines montane and valley populations = 147). All counts taken 9:00–11:00 A.M.

Costermans (1983) recorded the flowering periods of 124 *Acacia* species distributed through south-eastern Australia, and only 18 of them were not recorded in flower from August through October. Few species flower exclusively during summer and autumn.

INFLORESCENCE ANTHESIS

Inflorescences on the same branch or sharing the same raceme tend to flower acropetally or subacropetally (Bernhardt, 1982; Bernhardt et al., 1984). The staggering of the flowering of inflorescences on a raceme varies according to species. Inflorescences on a raceme of *Acacia pycnantha* flower subsynchronously. Consequently, individual shrubs tend to be mass-flowerers for two or three weeks. Less than half of the inflorescences on a raceme of *A. terminalis* may be found in flower over the functional lifespan of a raceme (Table 1). These small trees appear to remain in flower for up to a month and a half (Knox et al., 1985). The flowers comprising an *Acacia* inflorescence tend to open acropetally in long-spicate inflorescences, but they open synchronously-subsynchronously in globose heads (sensu Bernhardt, 1982; Bernhardt et al., 1984).

The receptive stigmas are the first structures to emerge and expand after the petals separate. At this time the stamens remain tightly clustered around the base of the ovary and the anthers are indehiscent. The lifespan of this "female phase" inflorescence appears to be dependent, in part, upon ambient moisture and temperature. The subsequent male phase is identified by the occlusion of the styles by the elongated staminal filaments and by anther dehiscence. Of course, the receptivity of the stigma changes from the female (pistillate) to male (staminate) phases, but this is discussed in Knox & Kenrick (this volume). Inflorescences tend to remain in the male phase for up to three to five days longer than they do in the female phase. Consequently, the number of male phase inflorescences on a tree or shrub tend to outnumber inflorescences still in the female phase (Table 1). The synchronicity of protogynous phases within a globular inflorescence of Australian *Acacia* contributes to the successful argument of Arroyo (1981) that the reproductive unit of a mimosoid taxon is the whole inflorescence and not the individual floret. Bagging experiments show that the rate of mechanical self-pollination (autogamy) has a low rate of success in the species of *Acacia* studied thus far (Bernhardt et al., 1984; Knox et al., 1985). Australian *Acacia* also show a high self-incompatibility index following controlled pollinations (Kenrick et al., 1982, 1984a, b; Kenrick & Knox, 1985; Knox & Kenrick, 1982). The mechanism of recognition and rejection is discussed by Kenrick & Knox (this volume).

III. Primary and Secondary Attractants

color and scent

Except for a single species confined to tropical Australia, the inflorescences of all continental and Tasmanian *Acacia* species appear white to orange-yellow. Floral pigments are probably restricted to carotenoids. There is no direct evidence for the presence of flavonoids, which combine with carotenoids to produce the bee-purple effect (K. Rowan, as cited by Bernhardt, 1982). It has long been understood that the conspicuous nature of an *Acacia* inflorescence is due almost exclusively to the coloration of the staminal filaments (Von Marilaun, 1903). In this respect the visual attractant of Australian *Acacia* parallels some sympatric genera in the Myrtaceae (e.g., *Darwinia, Melaleuca, Callistemon, Eucalyptus,* etc.). However, the filaments are not the only source of visual attraction in *Acacia*. The anthers may contrast with the filaments in shade. For example, in *A. mitchelii* the filaments are a light straw color but the anthers are bright golden-orange.

Inflorescence coloration varies intraspecifically. Pescott (ca. 1914) was among the first to record that shrubs of *Acacia paradoxa* (syn.: *A. armata*) differ in floral color from their siblings and maternal parent. *Acacia verticellata* is sufficiently polymorphic that changes in the density of florets and the actual shape of the inflorescence may accompany color variations (Willis, 1978). What local effect this may have on the fidelity of resident pollinators is unknown, although allopatric variations in the inflorescences of *A. terminalis* correlate with shifts in their pollinator spectra (see below).

Floral scent appears to be far more variable than color. Descriptions of the intense odor produced by some species has been listed by Bernhardt (1982). Some species offer an acrid stink, while the majority appear to produce a sweet fruity fragrance. Species like *Acacia myrtifolia* appear to combine both types of odors (Bernhardt, 1982). It is not surprising that Australian *Acacia* were incorporated into the European perfume and horticultural trades shortly after Australian explorations by the French (Ducker & Knox, 1982). Standard tests with neutral red suggest that osmorphores are located on the anthers and stigmas (Bernhardt, 1982; Bernhardt et al., 1985). It is true that the staminal filaments take up this stain after several hours of soaking, but this is probably due to permeability rather than the presence of volatile oils (M. Singh, pers. comm.).

Analyses of the volatile components in the flowers of three *Acacia* species not native to Australia yielded 114 compounds. The number of components varied between species, and it has been suggested that different floral foragers respond to different combinations of volatiles (Flath et al., 1983). Pilot studies at the Hormel Institute in Minnesota suggest that the floral odors of Australian *Acacia* are lower in intensity units and possess less than 25% of the number of components detected in the fragrances of *Acacia* species analyzed by Flath et al. (1983). Fatty acid esters appear to dominate the Australian *Acacia* species with sweet fruity fragrances, while acrid stinks are low in such compounds but have consistent levels of pyridine (R. Holman, unpublished).

edible rewards

Contrary to earlier descriptions of the flowers of Australian *Acacia* by competent naturalists, there appears to be no evidence that the florets contain nectaries, in contrast to the nectariferous inflorescences of *acacias* in South Africa (Robbertse, 1974) and North America (Simpson, pers. comm.). The "nectar" described in flowers of *A. leiocalyx* (Hawkeswood, 1983) appears to be nonsecretory silver trichomes that ornament the ovary (Kenrick, pers. comm.). The anthers of *A. bidwillii* bear pedicellate glands, but it is not known whether these glands are nectariferous (Bernhardt, 1982).

Virtually all Australian *Acacia* bear glands on their leaves or phyllodes (Boughton, 1981). In some species these glands secrete appreciable quantities of a fluid chemically comparable to floral

TABLE 2. Characteristics of the secretion of extrafloral nectar in four *Acacia* species.[a]

Characteristic of Nectary or Nectar	LO	MY	PY	TE[b]
Nectary secretes throughout flowering season[c]	+	+	+	+
Nectary a contrasting color from leaf or phyllode (yellow, crimson, maroon)	+	+	+	+
Nectary secretes >1 μl every morning[c]	–	–	–	+
Nectar hexose (glucose and fructose) rich[d]	?	–	+	–
Nectar sucrose rich or sucrose dominant[d]	?	+	–	+
Amino acid concentration >6.0 μmol/ml (log scale to base 2)[c]	?	+	+	+
Nectar collections by ants observed	+	+	+	+
Nectar collections by other insects (bees, flies, beetles, wasps) and/or birds observed	+	+	+	+

[a]Derived from Bernhardt (1982), Bernhardt & Walker (1985), and Knox et al. (1985).
[b]LO = *Acacia longifolia;* MY = *A. myrtifolia;* PY = *A. pycnantha;* TE = *A. terminalis.*
[c]Observations and nectar collections made 7:00–9:00 A.M.
[d]Sensu Baker & Baker (1983).

nectar while the plant is in flower. O'Dowd, as cited by Bernhardt (1982) and Majer (1978), contended that these extrafloral nectaries attract ant guardians which disrupt the activity of phytophagous insects. There is no reason to dispute such observations, as the same condition has been well documented in neotropical acacias (Janzen, 1974). However, virtually all major groups of floral foragers described below have been recorded taking extrafloral nectar from the glands of *Acacia* species (Lamont, 1978; Bernhardt & Walker, 1984; Knox et al., 1985). Extrafloral nectar may be secreted at dawn and through the early morning. Observations of nectar secretion in valley populations of *A. terminalis* suggested that nectar secretion decreased significantly by midday, permitting viscous or crystallized residues to accumulate in the glandular cups (Knox et al., 1985).

The quantity and chemistry of nectar varies between species (Table 2). The nectar of *Acacia pycnantha* is sucrose weak, but the nectar of *A. myrtifolia* is almost exclusively sucrose (Bernhardt, 1982). The proportion of sucrose to other sugars (sensu Baker & Baker, 1983) may vary significantly between populations belonging to the same species (Table 3). Extrafloral nectar tests positively for the presence of phenolic compounds and is often rich in amino acids. However, the concentration of amino acids is far lower than in the floral nectars of Old World *Erythrina* species (Baker & Baker, 1983; Knox et al., 1985).

Extrafloral nectar is not produced by all Australian *Acacia* species (Bernhardt, 1982; Bernhardt & Walker, 1984, 1985; Bernhardt et al., 1984). Furthermore, it is most unlikely that postpollination exudate on the stigma functions as a nectar substitute (Kenrick & Knox, 1981; Knox & Kenrick, 1982). Pollen is probably the primary reward offered by the majority of Australian acacias.

Polyads appear to lack copious deposits of pollenkitt (Fig. 1; Bernhardt, 1982). During the male phase these polyads are partially extruded from the anther sac upon dehiscence (Kenrick & Knox, 1979). Foragers remove polyads as a result of either passive contact (Knox et al., 1985) or by directly probing and scraping the anthers (Bernhardt et al., 1984). Adherence of the polyads to the forager combines aspects of the animal's vestiture and static electricity (Fig. 2; or see Buchmann, 1983).

IV. Floral Foragers vs. Polyad Vectors

Psyllidae and Lepidoptera

As *Acacia* is a dominant component of the Australian flora, most species support a dense and diverse fauna. Small, brushlike inflorescences often imply a trend towards generalist entomophily

TABLE 3. The comparative proportions of sugars in the extrafloral nectar of two populations of *Acacia terminalis*.

Sugar Proportion	Number of Plants from Each Population	Montane		Valley	
		Mean	Range	Mean	Range
Fructose	33	0.25	0.07–0.42	0.31	0.08–0.44
Glucose	33	0.28	0.11–0.48	0.33	0.05–0.52
Sucrose	33	0.46	0.16–0.82	0.38	0.10–0.88

Source of Variation	df	SS	MS	F
Fructose				
between Groups	1	0.05	0.05	9.1[a]
within Groups	64	0.35	5.46	
Glucose				
between Groups	1	0.10	0.10	0.01ns
within Groups	64	0.62	9.68	
Sucrose				
between Groups	1	0.20	0.20	8.0[b]
within Groups	64	1.60	0.02	

[a]Significant, $F_{0.005}$ (1, 64) > 8.49.
[b]Significant, $F_{0.01}$ (1, 64) > 7.08.

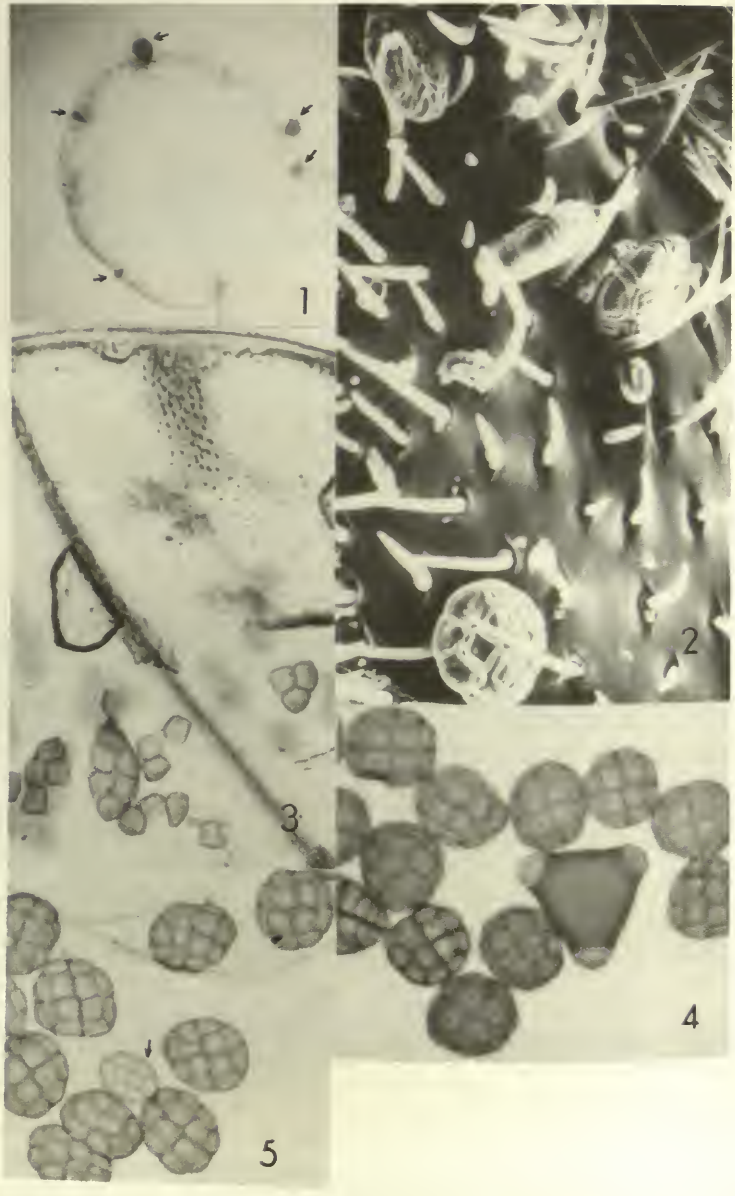
(sensu Faegri & van der Pijl, 1979). The difficulty with interpreting the vector spectrum of Australian *Acacia* species has been in separating the true pollinators (agents of cross-pollination) from mere visitors and/or floral predators. The removal of polyads by an insect taxon does not always imply that the same insect will regularly contact receptive stigmas. Psyllids, for example, are commonly found in the inflorescences of *Acacia*, particularly in those species that flower in winter (J. Kenrick, pers. comm.). Polyads or their remains are often collected on the wings of mature psyllids (Fig. 3; Bernhardt, 1982). However, as psyllid maturation requires the immature animals to devour the sexual organs of the flower, they are best regarded as floral predators (New, 1984).

The larvae of some Australian lepidoptera also destroy foliage and flower buds (New, 1984), but the majority of species do not exploit *Acacia* as a food plant. Technically, butterflies that forage on open inflorescences should be able to effect some cross-pollination. Hawkeswood (1985) has shown that fewer than half of the butterflies captured on *A. bidwillii* carried polyads. In the absence of floral nectar, these insects apparently do not remain on inflorescences long enough to obtain significant depositions of polyads. Of greater importance, the long legs of most butterflies prop up the body of the insects so that the anthers rarely contact the wide surfaces of the wings and abdomen while the butterfly attempts to forage.

COLEOPTERA

This huge order of insects highlights the problems inherent in distinguishing between phytophagous foragers and true pollinators. There is considerable intergradation between the two catagories, which will be clarified only with experimentation and further observation.

Beetles removed from *Acacia* inflorescences carry heavy loads of pollen (Hawkeswood as cited by Bernhardt, 1982; Hawkeswood, 1983). Some beetles strip away whole stamens and possibly consume carpels (Bernhardt et al., 1984; New, 1984). There are other points to consider. Beetles, while numerous, may not forage consistently over the entire flowering season on a single species of *Acacia* (Bernhardt et al., 1984). Although *Phlogistus* species (Carabidae) were often numerous



FIGURES 1–5. —1. Polyad of *Acacia pycnantha* photographed 30 seconds after application of Scarlet R stain. Beads of lipid-rich pollenkitt accumulate between exposed tips of pollen grains (indicated by arrows). —2. Scanning electron micrograph showing a portion of the ventral surface of the thorax of the colletid bee *Callomelitta* sp. with polyads of *Acacia terminalis* between hairs. Note absence of pollenkitt "gluing" polyads or insect hairs on cuticle. —3. Wing segment of *Psylla* sp. bearing remains of polyads of *Acacia baileyana*. —4. Portion of pollen load of female *Lasioglossum* sp. collected on *Acacia pycnantha* containing trioperculate grain of nectariferous *Hakea* sp. (Proteaceae). —5. Segment of branched scopal hair of *Lasioglossum* sp. with 8-grain polyad of *Acacia myrtifolia* (arrow) outnumbered by 16-grain polyads of *A. pycnantha*.

TABLE 4. Beetle taxa collected on the inflorescences of *Acacia* species.

Beetle Taxon	*Acacia* species on which Beetle Taxon Was Captured[a]										Number of Beetles
	BI	LO	ME	MI	MY	PA	PY	RR	RU	TE	
Alcmeonis pulchra	0	0	2	0	0	0	0	0	0	0	2
Aphneope sericata	0	0	1	0	0	0	0	0	0	0	1
Automolus depressus	0	0	0	0	0	0	0	0	2	0	2
Belidae	0	0	0	0	0	0	0	0	1	0	1
Cacochroa decorticata	1	0	0	0	0	0	0	0	0	0	1
Chromomea unicolor	0	0	0	0	0	1	0	0	0	0	1
Cleobora mellyimules	0	0	0	0	0	0	0	0	1	0	1
Cleridae	1	0	0	0	0	0	0	0	0	0	1
Clytus cortisi	9	0	0	0	0	0	0	0	0	0	9
Eleale pulchra	0	0	3	0	0	0	0	0	0	0	3
E. simplex	0	0	9	0	0	0	0	0	0	0	9
Eleale sp.	0	0	8	0	0	0	0	0	0	0	8
Glycyphana stolata	3	0	0	0	0	0	0	0	0	0	3
Heteromyx dimidiatus	0	0	0	0	0	1	0	0	0	0	1
Laius sp.	0	0	0	0	1	0	0	0	0	0	1
Lemidia subaenia	0	0	0	0	0	0	2	0	0	0	2
Liparetrus dicipennis	0	0	0	0	0	0	0	1	0	0	1
Melobasis obscurella	0	0	0	0	0	5	0	0	0	0	5
Melobasis spp.	0	0	1	0	1	1	0	0	0	0	3
Metriorrhynchus sp.	1	0	0	0	0	0	0	0	0	0	1
Oroderes humeralis	0	0	0	0	1	0	0	0	0	0	1
Pempsamacra dispersa	0	0	3	0	0	0	0	0	0	0	3
P. pygmaea	0	0	2	0	0	0	0	0	0	0	2
Phlogistus hintzi	0	0	0	0	1	0	2	0	0	0	3
Phlogistus spp.	0	0	0	0	7	0	0	0	0	0	7
Polystigma punctata	1	0	0	0	0	0	0	0	0	0	1
Rhinotia haemoptera	0	0	0	0	0	3	0	0	0	0	3
Rhyparida polymorpha	0	0	0	0	0	0	0	0	1	0	1
Sarothrocrepis civica	0	0	0	0	0	0	1	0	0	0	1
Stenoderus suturalis	0	0	0	0	0	0	0	0	3	0	3
Zenithicola crassus	1	0	0	0	0	0	0	0	0	0	1
Number of Beetle Taxa/*Acacia* species	7	0	8	0	5	5	3	1	5	0	

[a]BI = *Acacia bidwillii;* LO = *A. longifolia;* ME = *A. mearnsii;* MI = *A. mitchellii;* MY = *A. myrtifolia;* PA = *A. paradoxa;* PY = *A. pycnantha;* RR = *A. retinodes* var. *retinodes;* RU = *A. retinodes* var. *uncifolia;* TE = *A. terminalis.*

on *A. myrtifolia,* they could be collected only during the last two weeks of the flowering season and they foraged principally on inflorescences with dying, withered stamens. When the beetle taxa on ten *Acacia* taxa are compared, there appears to be little overlap of insect genera between *Acacia* species (Table 4). Excluding the genus *Phlogistus* and the buprestid *Melobasis,* there is little evidence to suggest that individual beetles or generations of the same beetle population show much fidelity to flowering *Acacia,* unlike some fly and bee taxa (Tables 5, 6). There is also the question as to whether beetles are effective agents of cross-pollination. If *Pyrgoides dryops* (Chrysomelidae) tend to remain on individual shrubs of *A. leiocalyx,* they tend to be instruments of self-pollination (Hawkeswood, 1983).

On the other hand, many genera of Coleoptera have modified mouthparts and digestive tracts for the consumption of pollen and nectar (Crowson, 1981). Many of these taxa are distributed in

TABLE 5. Fly taxa collected on the inflorescences of *Acacia* species.

Fly Taxon	*Acacia* Species on Which Fly Taxon Was Captured[a]									Number of Flies
	LO	ME	MI	MY	PA	PY	RR	RU	TE	
Calliphora stygia	3	0	0	7	1	3	0	0	0	14
C. vicina	0	0	0	1	0	0	0	0	0	1
Calliphora sp.	0	0	0	0	0	0	0	0	4	4
Calliphoridae	0	0	0	0	0	0	0	0	1	1
Ephydridae	1	0	0	0	0	0	0	0	0	1
Eristalis copiosus	0	0	0	0	0	0	0	2	0	2
E. punctatus	0	0	0	0	0	0	0	2	0	2
Hardimyia elongata	0	0	0	0	0	0	1	0	0	1
Heleomyzidae	0	1	0	0	0	0	0	0	0	1
Helina sp.	0	0	0	0	2	0	0	0	0	2
Incurviseta sp.	0	0	0	0	0	0	0	1	0	1
Lucila sp.	0	0	0	0	1	0	0	0	0	1
Melangyna viridicens	0	0	0	0	0	0	0	1	0	1
M. viridiceps	0	0	1	0	0	0	0	0	0	1
Melanostoma spp.	0	1	0	0	4	0	0	0	0	5
Microdon sp.	0	0	0	0	1	0	0	0	0	1
Musca vetustissimus	0	0	0	0	0	0	0	3	0	3
Muscidae	0	0	0	2	1	0	0	0	0	3
Psilota spp.	0	1	0	0	1	0	0	0	0	2
Pyrellia sp.	0	0	0	0	0	1	0	0	0	1
Rhagionidae	0	0	0	0	0	0	1	0	0	1
Rivellia sp.	1	0	0	0	0	0	0	0	0	1
Sarcophagidae	0	0	0	0	0	0	2	0	0	2
Senostoma sp.	0	0	0	0	0	0	0	1	0	1
Sphiximorpha breviscopa	0	0	0	0	0	0	1	0	0	1
Stomorhina subapicalis	0	0	0	0	0	0	0	6	0	6
Syrphidae	0	0	0	0	3	0	0	0	0	3
Syrphus damastor	1	15	0	27	15	14	0	0	0	58
Syrphus spp.	0	5	0	1	0	0	0	0	7	13
Tachinidae	0	0	0	0	0	0	1	0	0	1
Tricharene brevicornis	0	0	0	0	0	0	0	3	0	3
Triglyphus spp.	0	0	0	6	0	1	0	0	0	7
Xanthogramma grandicornis	0	0	0	0	0	0	0	28	0	28
Xylota sp.	0	0	0	0	0	0	0	0	1	1

[a]Abbreviations of *Acacia* species names as in Table 4.

Australia and belong to the families Buprestidae, Cleridae, and Chrysomelidae (Armstrong, 1979). These families seem well represented on the inflorescences of *Acacia* (Table 4).

The most convincing example of beetle pollination of an *Acacia* species that I've observed to date occurred in a population of *A. mearnsii* in northeastern Victoria. From 11:00 A.M. to 2:00 P.M. on hot sunny days, the inflorescences of these trees were covered with clerid beetles (*Eleale* spp.). which foraged on the anthers but did not damage carpels. They were observed "searching for anthers" on female phase inflorescences, and they flew from tree to tree. Twenty-one out of 26 beetles carried polyads of *A. mearnsii*. Over 61% of those beetles also carried the pollen of nectariferous Myrtaceae in association with the polyads of nectarless *A. mearnsii*.

DIPTERA

In direct contrast to the Coleoptera, the role of true flies in the crosspollination of Australian *Acacia* appears far easier to discern. Unlike the Coleoptera, taxa within the Diptera were collected

TABLE 6. Bee taxa collected on the inflorescences of *Acacia* species.

Bee Taxon	*Acacia* Species on Which Bee Taxon was Captured[a]									Number of Bees
	LO	ME	MI	MY	PA	PY	RR	RU	TE	
Anthophoridae										
Exoneura (Brevineura) spp.	0	4	0	0	0	0	0	0	0	4
Exoneura (Exoneura) spp.	0	18	0	0	0	0	2	0	11	31
										35
Apidae										
Apis mellifera	A[b]	1	1	5	4	A	A	A	A	11
Colletidae										
Amphylaeus sp.	0	0	0	0	0	0	0	0	1	1
Callomelitta perpicta	0	0	0	0	0	0	0	0	4	4
Callomelitta spp.	0	0	1	2	0	0	1	0	1	5
Euhesma spp.	0	0	0	0	0	3	8	0	0	11
Euryglossa spp.	1	0	0	0	2	0	0	0	0	3
Hylaeus sp.	0	0	0	0	0	0	1	0	0	1
Leioproctus metallescens	0	0	0	0	0	0	0	14	0	14
L. plumosus	0	0	0	0	0	0	0	1	0	1
Leioproctus (Euryglossidia) spp.	0	0	0	0	42	0	2	0	0	44
Leioproctus (Leioproctus) spp.	3	0	2	12	7	23	19	0	2	68
Trichocolletes sp.	0	0	0	0	1	0	0	0	0	1
										153
Halictidae										
Homalictus brisbanensis	0	2	0	0	0	0	0	10	0	12
H. demissus	0	0	0	0	0	0	1	0	0	1
H. dixoni	0	0	0	0	0	0	0	0	5	5
H. holochorus	1	0	0	0	0	0	0	0	0	1
H. megastigmus	0	0	2	0	7	0	1	0	0	10
H. oxoniellus	0	0	0	0	0	0	0	4	0	4
H. punctatus	0	0	0	1	0	0	1	0	0	2
Lasioglossum (Australictus) spp.	0	12	0	0	0	0	0	0	0	12
Lasioglossum (Austrerylaeus) spp.	0	0	0	0	0	0	0	0	1	1
Lasioglossum (Chilalictus) spp.	0	9	12	5	2	2	6	1	4	41
Lasioglossum (Parasphecodes) spp.	2	1	9	9	22	6	16	71	22	158
Nomia spp.	0	5	5	0	0	0	0	0	0	10
										257
Megachilidae										
Megachile sp.	0	0	0	0	0	0	0	2	0	
Number of bee taxa/*Acacia* species	5	8	7	6	8	5	12	6	9	
Total	7	52	32	34	87	34	58	103	51	458

[a]Abbreviations of *Acacia* species names as in Table 4.

[b]A = Abundant. Observed often but not collected.

on every *Acacia* taxon studied in Victoria. Furthermore, a far greater number of flies were collected on the inflorescences than were beetles (Tables 4, 5).

Excluding members of the genus *Calliphora* (Calliphoridae), the Syrphidae were the most commonly represented family (e.g., *Melangyna, Stomorhina, Syrphus, Triglyphus,* and *Xanthogramma*). Adult members of the family Syrphidae are known to eat pollen and are considered legitimate pollinators of other Australian angiosperms (Armstrong, 1979). They have often been observed and captured on taxa that offer pollen but lack floral nectar (Armstrong, 1979; Bernhardt, 1984; Bernhardt & Burns-Balogh, 1986). Six fly taxa were collected on more than one *Acacia* taxon (Table 5). This suggests that *Acacia* polyads may be an important component in the diet of some

adult flies and that individual flies or generations of the same fly population may follow the year-round floral phenology of the genus. However, the most commonly collected taxon, *Syrphus damastor*, is naturalized in Australia (K. Walker, pers. comm.) and was the only fly taxon on more than four species of *Acacia*. The impact of this fly species on the floral ecology of the Australian angiosperms has never been assessed. It still remains to be determined whether this ubiquitous species has displaced endemic flies.

Flies belonging to the family Syrphidae were most commonly observed on *Acacia* inflorescences between 9:00 A.M. and 12:00 M. They were usually the first insects collected on an *Acacia* in the morning. Unlike the other major groups of insect pollinators, syrphids were virtually the only insects active on inflorescences on wet, cool, cloudy days, and were observed foraging in light rains. Flies failed to distinguish between male and female phase inflorescences. Examination of insect bodies showed that polyads were deposited ventrally and on the proboscis.

HYMENOPTERA

Ants comprise the most diverse group of Hymenoptera in Australia. Different species may be encountered frequently on *Acacia* trees and shrubs at all hours, crawling on both vegetative and reproductive structures. However, there is no current evidence to suggest that ants disperse polyads (Lamont, 1978; Majer, 1978; Bernhardt, 1982). Furthermore, there is evidence to suggest that pollen deposited on the body of some ant taxa loses viability within a few minutes (Beattie, 1982).

Wasps were not collected on *Acacia* taxa in Victoria with the same frequency as were true flies (Tables 5, 7). Of 24 wasp taxa captured, only 29% were collected on more than one *Acacia* taxon (Table 7). No wasp taxon was collected on more than two *Acacia* species, and the wasps were rarely observed probing the inflorescences. Consequently, the proportion of wasps bearing polyads remained low. The number of wasps collected on any *Acacia* taxon does not reflect their ability to act as true pollinators. For example, less than a third of the wasps collected on the inflorescences of valley populations of *A. terminalis* carried polyads (Knox et al., 1985). Wasps appear to visit extrafloral nectaries first and forage secondarily on the inflorescences. Therefore, the *Acacia* species that secrete extrafloral nectar when they flower receive a wider diversity of wasps than those *Acacia* taxa that never secrete nectar (Tables 2, 7).

Bees remain the dominant hymenopterans that forage on *Acacia* inflorescences and they are probably the major group of insects on *Acacia* species (Table 6). At least one genus in each of the five families of bees distributed throughout Australia have been collected on *Acacia* (Table 6; Armstrong, 1979). Excluding the naturalized *Apis mellifera*, the most commonly collected bees belong to the two short-tongue families Colletidae and Halictidae. Apidae are poorly represented in Australia (Michener, 1979). Therefore, the comparatively few references to native *Trigona* species foraging on *Acacia* would seem highly predictable (Michener, 1965; Armstrong, 1979; Hawkeswood as cited by Bernhardt, 1982). However, the comparative absence of long-tongue Megachilidae on *Acacia* (Table 6) cannot be attributed merely to biogeography, as this bee family is relatively common throughout coastal Australia (Michener, 1979; Armstrong, 1979). Megachilid bees are typically associated with papilionoid legumes in Australia (Armstrong, 1979). Their 'boycott' of Australian *Acacia* species may reflect a foraging preference for the presence of floral nectar while they harvest pollen. Putatively nectariferous species of *Acacia* in Mesoamerica are regularly visited by megachilids (Janzen, 1974).

Of the 27 bee taxa collected on species of *Acacia*, slightly less than half were collected on more than one taxon (Table 6). Of all the major insect groups discussed previously, the Apoidea obviously show the greatest degree of abundance on *Acacia* over the flowering period of the genus in southeastern Australia. In Victoria, *Lasioglossum* (subg. *Parasphecodes;* Halictidae) have been collected on every *Acacia* species studied, and they are collected more frequently than any other bee taxon. *Leioproctus* species (Colletidae) visited all *Acacia* taxa except *A. mearnsii* (Table 6). How-

TABLE 7. Wasp taxa collected on the inflorescences of *Acacia* spp.

Wasp taxon	*Acacia* species on which wasp was captured[a]									Number of wasps
	LO[b]	ME	MI	MY[b]	PA	PY[b]	RR	RU	TE[b]	
Br aconide										
Apanteles sp.	1	0	0	0	0	0	0	0	0	1
Eumenidae										
Antamenes sp.	0	3	0	0	0	0	0	0	0	3
Pseudozethus sp.	0	0	0	0	0	0	0	0	5	5
Unidentified sp.	0	0	1	0	0	0	0	0	0	1
										9
Ichneumonidae										
Biconus sp.	0	0	0	1	0	0	0	0	0	1
Labium spp.	1	0	0	0	0	0	0	0	3	4
Lissopimpla excelsa	0	0	0	1	0	0	0	0	0	1
Lissopimpla sp.	0	0	0	0	0	0	0	0	2	2
										8
Pergidae										
Neourys sp.	0	0	0	0	0	1	0	0	0	1
Pompilidae										
Chirodamus sp.	0	0	0	0	0	0	0	0	1	1
Pompilius sp.	0	0	0	0	0	0	0	0	1	1
										2
Sphecidae:										
Cerceris antipodes	0	0	0	0	0	0	0	0	7	7
Cerceris spp.	0	0	0	0	1	0	0	0	6	7
Harpactophilus sp.	1	1	0	0	0	0	0	0	0	2
Rhopalum sp.	0	0	0	0	0	0	0	0	1	1
Sphex sp.	0	0	0	1	0	0	0	0	0	1
										18
Tiphiidae:										
Anthobosca sp.	0	0	0	0	0	0	0	3	2	5
Lophocheilus anilitatus	0	0	0	1	0	1	0	0	0	2
Phymatothynnus pygidalis	0	0	0	0	0	1	0	0	0	1
Phymatothynnus sp. nov. near nonilicornis	0	0	0	0	0	3	0	0	0	3
Phymatothynnus sp.	0	0	0	1	0	0	0	0	0	1
Rhagigester comparatus	0	0	0	0	0	2	0	0	0	2
Tachynomia moerens	0	0	0	0	0	2	0	0	0	2
Unidentified spp.	2	0	0	0	1	0	0	0	0	3
										19
Total number of wasps/*Acacia* sp.	5	4	1	5	2	10	0	3	28	58
Total number of wasp taxa/*Acacia* sp.	4	2	1	5	2	6	0	1	9	

[a]Abbreviations of *Acacia* species names as in Table 4.
[b]Secretes extrafloral nectar while flowering (Table 2).

ever, they were the most abundant foragers on *A. paradoxa* and *A. pycnantha*, outnumbering *Lasioglossum*.

Bees are the only floral foragers on *Acacia* that collect polyads to feed to their larvae. Polyads are actively removed from anthers by scraping, but species of *Lasioglossum* and *Leioproctus* also apply thoracic vibration. Bees have been frequently observed attempting to vibrate styles of female phase inflorescences. Polyads are transferred to the corbiculae of Apidae, but they are moistened and packed down with saliva and nectar. The other bee foragers on *Acacia* do not appear to moisten pollen, and the polyads are transferred to scopal hairs on the hindlegs and abdomen (Bernhardt,

1982; Bernhardt et al., 1984; Bernhardt & Walker, 1984, 1985). The caked and greasy texture of pollen loads on some *Acacia*-foraging bees is due to a mixture of pollen grains from co-blooming genera that bear copious deposits of lipid on their exines (e.g., *Hibbertia* (Dilleniaceae); Bernhardt, 1986).

VERTEBRATES

Turner (1982) reviewed the literature pertaining to reports of small wingless mammals that pollinate angiosperms. There is only one report of the marsupial *Trichosurus caninus* foraging on *Acacia*, and its efficacy as a pollinator is unknown (Breeden & Breeden, 1972).

There is now ample evidence, however, that some small birds might pollinate Australian acacias. Ford & Forde (1976) observed honeyeaters (Meliphagidae) foraging on the extrafloral nectar glands of *A. pycnantha*. As birds searched the secreting glands, their heads contacted the inflorescences in the axils of the phyllodes. The authors suspected that visible pollen on the birds' heads belonged to *A. pycnantha*, but they did not net birds to identify the pollen loads.

More convincing evidence for ornithophily has been presented by Kenrick et al. (1982) and Knox et al. (1985) in the autumnal flowering *A. terminalis*. The large crimson nectar glands on the leaf petioles brim with an average of 3.52 μl of nectar containing an average of 16% dissolved sugars during the morning hours. Passerines in the families Acanthizidae, Meliphagidae, and Zosteropidae actively forage for the extrafloral nectar and visit 2–4 trees during a foraging bout. When the bird removes nectar from a gland, its head contacts the inflorescences in the axils and polyads are deposited on the contour feathers and semiplumes of the head, neck, and shoulders. Pollen smears removed from these feathers contained up to 160 polyads.

VI. Dynamics of the Pollination Ecology of *Acacia*

MECHANISMS OF CROSS-POLLINATION

The successful transfer of polyads from one plant to the stigmas of another may occur by active or passive polyad collection. The majority of *Acacia* species studied thus far are cross-pollinated following active pollen collection by insect foragers. Insects bearing polyads attempt to forage on a female phase inflorescence for more polyads. This shows a trend towards pollination by deceit, defined specifically as automimicry. The polyad vector is unable to distinguish between flowers bearing polyads (male phase) and flowers that temporarily lack the reward (female phase). This is a common characteristic of diclinous plants in the neotropics (Dafni, 1984). Unisexual female flowers may mimic male flowers, as only the male flowers offer edible rewards. In the Australian *Acacia* species, however, automimicry is based on "temporal dicliny," as the short-lived female phase inflorescence offers the standard attractants but is devoid of accessible pollen. This explains the fidelity of certain insects to *Acacia* inflorescences. These mass-flowering shrubs and trees offer a disproportionately high number of male phase inflorescences to female phase inflorescences. The prospective vector always receives "partial reinforcement" for its foraging effort. The proportion of female phase inflorescences may vary over the flowering season of an individual plant, but female phase inflorescences do not outnumber inflorescences in the male phase and no inflorescence remains in the female phase permanently (Tables 1, 8).

Thus far, passive pollen collection has been shown only in *Acacia terminalis*. When pollinated by birds, these inflorescences represent a minor obstruction but not a source of edible rewards. The birds do not probe the inflorescences; to effect cross-pollination a receptive stigma must probe or comb the bird *after* anthers have powdered the plumage (Knox et al., 1985). This unusual syndrome may have evolved independently several times in the South Pacific and the Neotropics. Extrafloral

TABLE 8. Flowering behavior of *Acacia pycnantha* over a 12-week flowering season.

		Flowering seasons division[a]		
Inflorescence characteristic	*n*	Early	Mid	Late
Mean ratio of the frequency of female phase inflorescences on a raceme	60	0.33	0.12	0.19
Source of variation	df	SS	MS	F
Between groups	2	0.44	0.22	1.69[b]
Within groups	57	7.46	0.13	

[a]Each seasonal division is divided into four weeks, with five racemes counted each week.
[b]Not significant.

secretions are also the reward for pollinators in some Araceae and Euphorbiaceae (Simpson & Neff, 1981). Edible bracts attract birds and bats that pollinate the tiny rewardless flowers of *Freycinetia* (Pandanaceae) (Cox, 1983) and *Boerlagiodendron* (Araliaceae) (Faegri & van der Pijl, 1979). Obviously, passive pollen collection in *A. terminalis* represents a condition derived from an ancestor pollinated via automimicry. It must be emphasized that active pollen collection still occurs in this species and, in certain populations, this mode of pollination by bees precedes ornithophily in importance.

POLYLECTIC FORAGING AND "*ACACIA* BEES"

In his review of flowers that offer pollen as their only reward, Vogel (1978) was the first to interpret accurately the Australian *Acacia* inflorescence as a Papaver Type: one where densely massed florets bear many long stamens and where polyads are retained on or in the small terminal anthers until removed by female bees. Of course, this interpretation must be expanded to include beetles and flies, but the sheer importance of bee pollinators requires deeper analysis. Only two decades ago, Michener (1965) noted that *Acacia* seemed of little importance to Australian bees.

Although recent collections have greatly expanded our knowledge of the number of bee taxa that might forage on *Acacia*, such a list does not reflect the true diversity of Apoidea in southeastern Australia (Table 6 vs. Michener, 1965; or Armstrong, 1979). In fact, it must be further emphasized that the restricted diversity of Apoidea on *Acacia* throughout southeastern Australia does not represent a trend towards oligolecty (sensu Michener, 1979). The mode of floral presentation and restricted edible reward offered by Australian *Acacia* inflorescences would appear to discourage oligolectic foraging. The exposed, densely compacted anthers bear partially extruded polyads; so pollen is accessible to virtually all female bees collecting material for nest lining or food for larvae. However, the individual florets offer no immediate source of chemical energy for the forager, as most *Acacia* species lack extrafloral nectar.

Consequently, foraging bees must either leave their nests with full supplies of chemical energy prior to foraging exclusively on *Acacia* inflorescences or they must supplement foraging bouts to nectarless inflorescences with visits to nectariferous plants. Naturalized *Apis* workers exploit the former pattern of foraging, as they may rely on honey reserves. Pollen loads of *A. mellifera* workers collected on *Acacia* are usually pure loads of polyads (Kenrick et al., 1982; Bernhardt et al., 1984). In fact, a division of labor is observed on *Acacia terminalis*, with some *A. mellifera* taking only nectar from extrafloral glands while other workers forage for polyads exclusively. It is possible that the same pattern occurs in endemic *Trigona*, as they are eusocial Apidae.

However, the majority of bee taxa collected on *Acacia* employ the latter pattern of "punctuated foraging." Virtually all the bee genera collected on *Acacia* more than once are known to exploit a

TABLE 9. Flowering patterns of *Acacia* species as correlated within the taxonomic diversity and foraging behavior of the bees.

Flowering period and *Acacia* species	Number of bee taxa on *Acacia* species	Number of bees bearing *Acacia* polyads[a]	Ratio[b]
Midwinter–early spring			
A. longifolia	5	22 (9)	0.40
A. pycnantha	5	41 (9)	0.21
Mid winter–mid spring			
A. myrtifolia	6	29 (16)	0.55
Early spring–late spring			
A. paradoxa	8	96 (50)	0.52
Late spring			
A. mearnsii	8	59 (45)	0.91
Early summer–mid summer			
A. mitchellii	7	32 (25)	0.78
Early summer–late summer			
A. retinodes var. retinodes	12	67 (56)	0.80
A. retinodes var. uncifolia	8	121 (77)	0.63
Autumn–early winter			
A. terminalis	10	50 (16)	0.32

[a]The first number in the column refers to the total number of bees caught on the particular *Acacia* species that carried polyads of *Acacia* on their bodies. The second number, in parentheses, refers to the total number of bees that carried *Acacia* polyads plus the pollen of other genera.

[b]Ratio = Number of bees that carried *Acacia* polyads plus pollen of other genera/total number of bees that carried *Acacia* polyads.

wide range of pollen sources (Armstrong, 1979). Polylectic foraging is the rule and not the exception in the genera *Leioproctus* and *Lasioglossum* (Bernhardt, 1982; Bernhardt et al., 1984; Bernhardt & Walker, 1984, 1985; Knox et al., 1985; Bernhardt, 1986a, 1987; Bernhardt & Burns-Balogh, 1986). If bees must break their foraging bouts on nectarless shrubs in order to obtain energy by visiting nectariferous taxa, natural selection should favor pollen foraging on the nectariferous flowers, provided pollen flow is equally copious and nutritious for larvae.

The number of bees collected on *Acacia* inflorescences bearing polyads mixed with the pollen of other taxa increases from late winter through late summer (Table 9). As the ambient temperature of semixeric habitats increases, the number of bee taxa should also increase (Moldenke, 1976; Michener, 1979); this occurs with a corresponding increase in the sheer density of nectariferous plants, especially Australian Myrtaceae (Bernhardt & Walker, 1984; Bernhardt, 1987). Consequently, more bees bearing homogenous loads of polyads are caught on acacias flowering in autumn and winter. These "cool weather" acacias (e.g., *A. terminalis*, *A. longifolia*, and *A. pycnantha*) bear functional extrafloral nectaries (Table 2). This appears to support polylectic bee guilds during seasons when the community nectar flow is poor (Table 9).

The number of pollen taxa identified in the scopae of those bees collected on *Acacia* inflorescences appears to be directly proportional to the diversity and density of the local flora. Bees that foraged on *A. mitchellii* in the Brisbane Ranges carried half the number of pollen taxa identified on bees collected on *A. myrtifolia*. *Acacia myrtifolia* flowered earlier in the season in the Brisbane Ranges during the month of intense vernal diversity (Bernhardt & Walker, 1984). *Acacia retinodes* var. *retinodes* and var. *uncifolia* flower concurrently but in widely divergent and isolated habitats. Bees collected on var. *uncifolia* carried pollen of naturalized Solanaceae and Compositae that invade disrupted coastal sites, but pollen of these other two families was absent on bees taken from inflorescences of var. *retinodes* in inland montane sites (Bernhardt et al., 1984; Bernhardt & Walker, 1985).

TABLE 10. Comparative frequencies of pollen from other spermatophyte families as identified on bees carrying *Acacia* polyads.

Spermatophyte family[a]	Number of bees bearing *Acacia* polyads mixed with pollen of other families captured on each *Acacia* species[b]									Total
	LO	ME	MI	MY	PA	PY	RR	RU	TE	
Compositae + −[c]	0	4	0	0	3	1	6	26	0	40
Dilleniaceae−	0	0	3	0	4	1	0	0	0	8
Epacridaceae +[c]	0	0	0	1	3	1	0	0	3	8
Leguminosae(Papilioinoidae) +	1	4	0	0	23	0	0	0	0	28
Liliaceae s.l. + −[c]	0	6	0	0	2	0	0	0	0	8
Myrtaceae +[c]	2	47	19	2	26	0	71	43	15	225
Pinaceae−	9	0	0	0	1	1	0	0	0	11
Pittosporaceae +[c]	0	0	0	0	0	0	2	0	0	2
Proteaceae +[c]	0	0	0	4	0	3	0	0	0	7
Rhamnaceae +[c]	0	0	0	12	2	4	0	0	0	18

[a] + = bees bearing pollen of genera with nectariferous flowers; − = bees bearing pollen of genera with nectarless flowers.

[b] Abbreviations of *Acacia* species names as in Table 4.

[c] Family contains one or more genera in which floral nectar is presented on a shallow perianth or retained in a short tube.

The majority of bees carrying polyads mixed with pollen of other angiosperms carry the pollen of nectariferous taxa in their scopae (Table 10). As most of the bee taxa on *Acacia* species belong to short-tongue genera in Australia, it is not surprising that they visit flowers with shallow nectaries (Table 10). Species of Myrtaceae tend to dominate the nectar flora of southeastern Australia, and the bees exploit the shallow-bowl flowers of *Eucalyptus*, *Leptospermum*, and *Melaleuca* more often than the genera of other families. Papilionoid legumes offer the only nectariferous flowers patronized by "acacia bees" that lack the characteristic short-tube or shallow-dish perianth (Table 10). Nevertheless, female bees may incorporate several different taxa into a foraging bout that includes an *Acacia* species (Fig. 4). *Lasioglossum* (Parasphecodes) carrying up to five pollen taxa in their scopae have been collected on *Acacia* (Bernhardt & Walker, 1984, 1985).

Bees that forage on nectarless *Acacia* species may also forage on the nectarless flowers of other taxa during the same foraging bout (Table 10). Such pollen loads usually include the pollen of at least one nectariferous taxon, especially Myrtaceae and Compositae. *Lasioglossum* (*Chilalictus*) spp. appear to be the major foragers on nectarless *Hibbertia* spp. (Dilleniaceae). When *Acacia* and *Hibbertia* species overlap, *Lasioglossum* (*Parasphecodes*) species are collected more often on *Acacia*, while *Lasioglossum* (*Chilalictus*) spp. are collected most commonly on *Hibbertia* (Bernhardt & Walker, 1984, 1985; Bernhardt, 1984, 1986, 1987).

Therefore, we cannot speak of "acacia bees" as other authorities commonly refer to clover bees, orchid bees, or squash and gourd bees. The morphological coevolution that has wed some *Trifolium* species to *Bombus* (Free, 1970) is not apparent between Australian *Acacia* and its bee guild. Naturalized *A. mellifera* appear to be preadapted as pollinators of Australian *Acacia*, as workers are also deceived by the automimicry of the inflorescences. Certainly, *Leioproctus* and *Lasioglossum* are the dominant pollinators, but the floral and polyad presentation of *Acacia* in Australia does not exclude foraging and subsequent cross-pollination by other bee taxa. Instead, the nectarless flowers encourage bees to maintain polylectic habits. Long-tongue anthophorids in the genus *Exoneura*, and the large-bodied *Callomelitta* spp. (Colletiidae) effectively pollinated *A. mearnsii* and *A. terminalis* in the absence of *Leioproctus* (Table 6). One needs to compare the *Acacia* system against that expressed by epidendroid orchids in the Neotropics, for example. Floral morphology and scent

modifications in the orchids restrict successful pollination to a comparatively narrow spectrum of euglossine bees (Dressler, 1981). Environmental pressures leading to fluctuations in the relative density and diversity of pollinator guilds will have a more immediate, deleterious effect on reproductive success in specialized bee–flower interactions than in the generalist system of Australian *Acacia*.

The bee fauna of an *Acacia* species appears to be based more on independent variation in bee demographics and bee phenology than on specific canalization of floral morphology or attractant/reward chemistry. For example, during the severely prolonged drought of spring 1982, *Leioproctus* (*Leioproctus*) spp. were commonly collected on *Acacia* spp. in the Brisbane Ranges. The number of bees collected on inflorescences declined from July to late January so that *Leioproctus* (*Leioproctus*) spp. were the dominant pollinators of *A. pycnantha*, but the same subgenus was represented nominally on *A. mitchellii* (Bernhardt & Walker, 1984). *Acacia mitchellii*, a late-flowering shrub (Table 9), was pollinated almost exclusively by halictid bees. During the wet cool spring of 1984, *Leioproctus* (*Leioproctus*) was never captured, but *Leioproctus* (*Euryglossidia*) spp. dominated the inflorescences of *A. paradoxa* in September and October (Tables 6, 9). Conversely, species in the *Euryglossidia* subgenus were absent during the 1982 drought (Bernhardt, 1986).

INTERSPECIFIC COMPETITION AND INTRASPECIFIC VARIATION

The pattern of generalist entomophily in most Australian *Acacia* species suggests that the fidelity of pollinators may also fluctuate according to overlapping floral phenologies. As reported previously, different subgenera of *Lasioglossum* forage preferentially on either *Acacia* or *Hibbertia* when the two dicot genera flower at the same time in the same habitat (Bernhardt & Walker, 1984; Bernhardt, 1984, 1986).

Interspecific competition for pollinators may also be intrageneric. Bees forage selectively on *Acacia pycnantha* in preference to *A. myrtifolia*. It is only after the flowering season of *A. pycnantha* that bees forage exclusively on *A. myrtifolia* (Bernhardt, 1982). The collection of polyads requires that adult bees expend energy in flight and while scraping and vibrating anthers. Inflorescences of *A. pycnantha* and *A. myrtifolia* have different numbers of flowers but similar numbers of stamens per inflorescence (Bernhardt & Walker, 1984). The 16-grain polyad of *A. pycnantha* has twice the volume as the 8-grain polyad of *A. myrtifolia* (Fig. 5). Bees are able to discriminate between the two species of *Acacia* due to their different primary attractants (Bernhardt, 1982; Bernhardt & Walker, 1984). Consequently, the bees conserve their own energy resources by foraging prefentially on *A. pycnantha*, which offers a larger reward for less effort (Bernhardt & Walker 1984).

Can fluctuations in the diversity of pollinators exert sufficient selective pressure to lead to intraspecific variation in a species of *Acacia*? Does such intraspecific variation lead to incipient speciation? Visitation in Australian *Acacia* appears so plastic that one would assume that a large group of pollinators could be substituted for another over the range of the *Acacia* species without significantly altering disjunct gene pools. The primary pollinators of *Acacia* are generalist foragers, and the presentation of inflorescences does not really restrict access to any insect that requires pollen, regardless of body size or foraging behavior.

Major variation in the guild of pollinators may, under unusual circumstances, lead to incipient speciation in *Acacia terminalis* (Kenrick et al., 1987). Montane and valley populations of this species are separated by less than 20 km. However, the presentation of primary and secondary attractants in both populations of *A. terminalis* remains divergent and distinctive (Tables 3, 11). The number of inflorescences per raceme, gland morphometrics, and nectar chemistry remain statistically significant (Table 3; Kenrick et al., 1987). R. Holman (pers. comm.) has analyzed the floral fragrances of both populations, and the fragrance produced by the valley population remains lower in individual components and intensity units compared with the montane populations.

TABLE 11. Qualitative and significant quantitative differences dividing the pollination syndrome of montane and valley populations of *A. terminalis*.

Characteristic	Montane	Valley
Phenology		
Flowering season[a]	2–4	4–7
Pod maturation/dehiscence[a]	2–3	12–1
Inflorescences		
Number of inflorescences/raceme	±6	±19
Inflorescence color	white–buff	yellow
Style length	short	long
Extrafloral nectary	small, circular	large, oblong
Nectar		
Grams sugar/1 μl	0.14	0.20
Proportion of sucrose	0.46	0.35
Proportion of fructose	0.25	0.31
Amino acids (ninhydrin scale)	+200 μg/ml	+500 μg/ml
Detectable phenols	0.03	0.25
Major pollinators	anthophorid, halictid bees, syrphid flies	Passerines, hallictid bees

[a]Months listed as numbers, with January = 1, etc.

A clue to this divergence lies in the differing spectrum of pollinators. Montane populations are pollinated primarily by long- and short-tongue families of bees; passerines and short-tongue bees dominate the valley populations. Strong floral fragrances are not generally indicative of bird-pollinated plants, but hexose-rich nectars are consistent with plants pollinated by both passerines and short-tongue bees (Faegri & van der Pijl, 1979; Baker & Baker, 1983). Long styles in the inflorescences of valley populations are more likely to contact birds brushing against the inflorescences in their attempts to reach the extrafloral nectaries. Bees forage actively for pollen on inflorescences, and amassing too many open inflorescences on the same raceme may encourage bee-mediated self-pollinations in montane habitats.

Therefore, while *Acacia* species in Australia tend to receive a wide range of floral foragers, the dominance of specific vectors varies intra- and interspecifically. In most populations the spectrum of pollinators emphasizes Apoidea, but beetles (e.g., *A. mearnsii*), passerines (*A. terminalis*), and flies (*A. myrtifolia*) remain important contributors to cross-pollination, especially when bee demographics and phenology fluctuate or sympatric *Acacia* species must compete for the same guild of polylectic bees.

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References

Armstrong, J. A. 1979. Biotic pollination mechanisms in the Australian flora—a review. New Zealand J. Bot. 17: 467–508.

Arroyo, M. T. K. 1981. Breeding systems and pollination in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanical Gardens, Kew.

Baker, H. G. & I. Baker. 1983. Floral nectar sugar constituents in relation to pollinator type. Pp. 117–141 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York.

Beattie, A. J. 1982. Effects of ants on pollen activity. Pp. 37–41 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors), Pollination '82. Plant Cell Biology Research Centre, Univ. of Melbourne.

Bernhardt, P. 1982. Insect pollination of Australian *Acacia*. Pp. 84–101 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors), Pollination '82. Plant Cell Biology Research Centre, Univ. of Melbourne.

———. 1984. The pollination biology of *Hibbertia stricta* (Dilleniaceae). Pl. Syst. Evol. 147: 267–277.

———. 1986. Bee-pollination in *Hibbertia fasciculata* (Dilleniaceae). Pl. Syst. Evol. 152: 231–241.

———. 1987. A comparison of the diversity, density, and foraging behavior of bees and wasps on Australian *Acacia*. Ann. Missouri Bot. Gard. 75: 42–50.

——— & P. Burns-Balogh. 1986. Floral mimesis in *Thelymitra nuda* R. Br. Pl. Syst. Evol. 151: 187–202.

——— & K. Walker. 1984. Bee foraging on three sympatric species of Australian Acacia. Int. J. Entomol. 26: 322–330.

——— & ———. 1985. Insect foraging on *Acacia retinodes* var. *retinodes*. Int. J. Entomol. 27: 97–101.

———, J. Kenrick & R. B. Knox. 1984. Pollination biology and the breeding system of *A. retinodes* (Leguminosae: Mimosoideae). Ann. Missouri Bot. Gard. 71: 17–29.

Boughton, V. H. 1981. Extrafloral nectaries of some Australian phyllodineous acacias. Austral. J. Bot. 29: 653–664.

Breeden, S. & K. Breeden. 1972. Australia's South East. A Natural History of Australia, 2nd edition. Collins, Sydney.

Buchmann, S. L. 1983. Buzz pollination in angiosperms. Pp. 72–114 *in* C. E. Jones & R. J. Little (editors), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York.

Costermans, L. 1983. Native Trees and Shrubs of Southeastern Australia. Rigby Publishers, Australia.

Cox, P. A. 1983. Extinction of the Hawaiian avifauna resulted in a change of pollinators for the *ie ie*, *Freycinetia arborea*. Oikos 41: 195–199.

Crowson, R. A. 1981. The Biology of The Coleoptera. Academic Press, London.

Dafni, A. 1984. Mimicry and deception in pollination. Ann. Rev. Ecol. & Syst. 15: 259–278.

Dressler, R. L. 1981. The Orchids, Natural History and Classification. Harvard Univ. Press, Cambridge, Massachusetts.

Ducker, S. C. & R. B. Knox. 1982. The Australian history of *Acacia* Miller and its pollen. Pp. 74–83 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors), Pollination '82. Plant Cell Biology Research Centre, Univ. of Melbourne.

Faegri, K. & L. van der Pijl. 1979. Principles of Pollination Ecology, 3rd edition. Pergamon, Oxford & New York.

Flath, R. A., R. T. Mon, G. Lorenze, C. J. Whitten & J. W. Mackley. 1983. Volatile components of *Acacia* spp. blossoms. J. Agric. Food Chem. 31: 1167–1170.

Ford, H. A. & N. Forde. 1976. Birds as possible pollinators of *Acacia pycnantha*. Austral. J. Bot. 24: 384–395.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, London.

Hawkeswood, T. H. 1983. Observations on *Pyrgoides dryops* (Blackburn) Coleoptera: Chrysomelidae, a pollen-feeding beetle on *Acacia leiocalyx* (Domin.) Pedley, at Brisbane, south-east Queensland. Victoria Naturalist 100: 156–158.

———. 1985. The role of butterflies as pollinators of *Acacia bidwilii* (Mimosaceae) at Townsville, Northern Queensland. Austral. J. Bot. 33: 167–73.

Janzen, D. H. 1974. Swollen-thorn acacias of Central America. Smithsonian Contr., Bot. 13.

Kenrick, J. & R. B. Knox. 1979. Pollen development and cytochemistry in some Australian species of *Acacia*. Austral. J. Bot. 27: 412–427.

_____ & _____. 1981. Structure and histochemistry of the stigma and style of some Australian species of *Acacia*. Austral. J. Bot. 29: 733–745.

_____ & _____. 1985. Self-incompatibility in the nitrogen fixing tree, *Acacia retinodes:* quantitative cytology of pollen tube growth. Theor. Appl. Genet. 69: 481–488.

_____, V. Kaul & R. B. Knox. 1984a. Self-incompatibility in *Acacia*—a pre- or post-zygotic mechanism? Pp. 146–153 *in* E. G. Williams & R. B. Knox (editors), Pollination '84. Plant Cell Biology Research Centre, Univ. of Melbourne.

_____, R. Marginson, G. Beresford & R. B. Knox. 1982. Birds and pollination of *Acacia terminalis*. Pp. 102–109 *in* E. G. Williams, R. B. Knox, J. H. Gildbert & P. Bernhardt (editors), Pollination '82. Plant Cell Biology Research Centre, Univ. of Melbourne.

_____, P. Bernhardt, R. Marginson, G. Beresford & R. B. Knox. 1984b. *Acacia* breeding systems. P. 210 *in* E. G. Williams & R. B. Knox (editors), Pollination '84. Plant Cell Biology Research Centre, Univ. of Melbourne.

_____, _____, _____, _____, _____, I. Baker & H. G. Baker. 1987. Pollination-related characteristics in the mimosoid legume *Acacia terminalis* (Leguminosae). Pl. Syst. Evol. 157: 49–62.

Knox, R. B. & J. Kenrick. 1982. Polyad function in relation to the breeding system of *Acacia*. Pp. 411–418 *in* D. Mulcahy & E. Ottaviano (editors), Pollen Biology. Elsevier, North-Holland.

_____, _____, P. Bernhardt, R. Marginson, G. Beresford, I. Baker & H. G. Baker. 1985. Extra-floral nectaries as adaptations for bird pollination in *Acacia terminalis*. Amer. J. Bot. 72: 1185–1196.

Lamont, N. 1978. Extra floral nectaries in Australian plants with special reference to *Acacia*. Mulga Res. Center Annual Rep. 15–18.

Majer. J. D. 1978. The possible protective function of extra floral nectaries of *Acacia saligna*. Mulga Res. Center Annual Rep. 2: 31–39.

Michener, C. D. 1965. A classification of the bees of the Australian and South Pacific regions. Bull. Amer. Mus. Nat. Hist. 130: 1–362.

_____. 1979. Biogeography of the bees. Ann. Missouri Bot. Gard. 16: 277–347.

Moldenke, A. R. 1976. Evolutionary history and diversity of the bee faunas of Chile and Pacific North America. Wassman J. Biol. 34: 147–778.

New, T. R. 1984. A Biology of Acacias. Oxford Univ. Press, in association with Latrobe Univ. Press, Melbourne, Australia.

Pedley, L. 1978–1979. A revision of *Acacia* (Mill.) in Queensland. Austrobaileya 1: 17–315.

Pescott, E. E. ca. 1914. The Native Flowers of Victoria. Georgia Robertson & Co., Melbourne, Australia.

Pryor, L. D. & L. A. S. Johnson. 1971. A Classification of The Eucalypts. Australian National Univ., Canberra, Australia.

Robbertse, P. J. 1974. The genus *Acacia* in South Africa II with special reference to the morphology of the flower and inflorescence. Phytomorphology 24: 1–15.

Simpson, B. B. & J. L. Neff. 1981. Floral rewards: alternatives to pollen and nectar. Ann. Missouri Bot. Gard. 68: 301–322.

Turner, V. 1982. Non-flying mammal pollination: an opportunity in Australia. Pp. 110–121 *in* E. G. Williams, R. B. Knox, J. H. Gilbert & P. Bernhardt (editors). Pollination '82. Plant Cell Biology Research Centre, Univ. of Melbourne.

Vogel, S. 1978. Evolutionary shifts from reward to deception in pollen flowers. *In* A. J. Richards (editor), The Pollination of Flowers by Insects. Linn. Soc. Symp. No. 6. Academic Press, London.

Von Marilaun, A. K. 1903. The Natural History of Plants, Volume II, The History of Plants. Gresham Publishing Co., London.

Willis, J. H. 1978. A Handbook to Plants In Victoria, Volume II, Dicotyledons. Melbourne Univ. Press, Melbourne.

Zapata, T. R. & M. T. K. Arroyo. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10: 221–230.

THE POPULATION BIOLOGY OF AN ANNUAL TEXAS LUPINE

Barbara A. Schaal[1]

Abstract

The population biology of an annual species of Lupine, *Lupinus texensis*, has been extensively studied. Pollination within populations is not at random, neither within inflorescences nor among plants. Studies of gene flow indicate that both the pollen and seed component of gene dispersal is geographically restricted within populations. Crossing tends to be among plants in close spatial proximity. Limited gene flow subsequently leads to genetic subdivision and isolation by distance within populations. Populations of *Lupinus texensis* show significant spatial genetic heterogeneity of allozyme frequencies. Population substructure can in turn lead to varying levels of inbreeding within populations, depending on the distance between parental plants. *Lupinus* has a mixed mating system; pollinator foraging leads to both selfed and outcrossed seed. Individuals produced by outcrossing have a greater fitness than do individuals produced by inbreeding. Outcrossed plants have significantly higher survivorship, longer life spans, and greater fecundity than do inbred plants. The effects of inbreeding depression are, however, mediated by maternal effects that are predominant in influencing fitness early in the life cycle of the plants.

I. Introduction

Population biology is a broad field covering several disciplines, including population ecology, genetics, and microevolution. Currently, research in plant population biology centers around two major themes, the numerical dynamics of populations and the genetic processes that occur within them. This latter area of population genetics deals with the levels of genetic variation and the factors influencing the levels and apportionment of variation. The study of genetic variation has been central to population biology, since it forms the basis of future evolutionary change. In plant species, the levels and distribution of genetic variation are strongly influenced by the relationships between plants and their pollinators.

II. Pollination Biology and Gene Flow

Legumes show a high level of coadaptation with their pollinators. There are well-documented examples of the intricate relationships between the legume flower and its pollinators (Arroyo, 1981; Schrire, this volume). Here I discuss the consequences of this plant–pollinator relationship for the genetic structure of plant populations. The pattern of pollinator foraging directly influences the crossing relationships within populations. If pollinators forage widely, gene migration will occur over large distances and populations will be homogeneous, with little genetic differentiation between them. Conversely, if pollinator foraging is very restricted, gene flow will be limited and one may expect significant local differentiation within populations as well as large genetic distances between populations. Likewise, the mating system and the pattern of pollinator foraging on an individual inflorescence or plant will influence gene migration.

Much of our work on genetic population structure has been with *Lupinus texensis*, an endemic winter annual of Texas, U.S.A. It is a conspicuous member of the spring flora, forming large

[1]Department of Biology, Washington University, St. Louis, Missouri 63130, U.S.A.

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TABLE 1. Genetic variation in *Lupinus texensis* (from Babbel & Selander, 1974).

Number of alleles/locus	3.12
Mean heterozygosity	0.36
Genetic identity	0.957

populations which often contain thousands of plants. *Lupinus texensis* has a typical papilionoid flower. It is bilaterally symmetrical and has a banner with a central spot which turns from white to red as the flower ages. The pollinators of these lupines are bees, which trip the flower mechanism to obtain large quantities of orange pollen. The morphology of the flower necessarily limits pollination to bees, which are in turn limited in how they forage on an inflorescence. Old flowers have a red banner spot and are seldom visited by bees; little pollen remains in the flower at this stage and what does remain is white, with a powdery consistency rather than the orange, sticky pollen of fertile flowers (Schaal & Leverich, 1980). Bees forage on the white banner spot flowers from the bottom to the top of the inflorescence. The flowers are protandrous, and so there is a mechanism to avoid extensive self-fertilization. Nonetheless, plants will set self-seed, both in the field and in the greenhouse. Pollinators forage in *Lupinus texensis* populations in an energy-efficient manner. Pollinator moves are not at random; instead there is a very high proportion of moves to the closest plant. Thus, the pollination system places constraints on the migration of genes within a lupine population. The probability of two plants crossing is strongly influenced by the pattern of pollinator foraging and by the fertility relationships within and between plants, factors which influence the genetics of lupine populations.

The pattern of pollinator foraging within populations of *Lupinus texensis* bears consequences on gene migration, which was studied in an experimental population (Schaal, 1980). Bees are capable of a high level of discrimination; therefore, the markers chosen to trace gene migration must not alter foraging patterns. Allozyme markers were used to track the migration of genes within an experimental population of lupines. Allozymes, alternate allelic forms of enzymes, are polymorphic in most plant populations, and in *Lupinus texensis* up to seven alleles occur at some loci (Table 1). The body of the experimental population consisted of plants that were homozygous for a phosphoglucose isomerase allele with an intermediate speed of migration. The margin of the population and the center of the population consisted of plants that were homozygous for a slow- and fast-migrating form of *PGI-1*, respectively. The plants were placed in a hexagonal array in an experimental garden and the foraging pattern of bees scored. The resulting F_1 seeds were harvested, germinated, and the genotype at the *PGI-1* locus determined. Episodes of gene migration were detected by the presence of heterozygous progeny.

The mean migration of alleles was 1.82 m; the mean foraging distance of the bee pollinators was 0.97 m. Such studies have been repeated in several plant species (Ennos & Clegg, 1983; Levin, 1981), and the results seem to be fairly consistent. Figure 1 shows the relationship between actual gene flow and pollinator foraging. In general, pollinators tend to forage in a highly restricted manner where most foraging moves are to the nearest-neighbor plant. The measures of gene migration are usually greater than the foraging distribution would indicate. Such a discrepancy between pollinator foraging distribution and actual gene migration is the result of pollen carryover. Pollinators do not usually deposit all of the pollen that they pick up from a given plant on the next one they encounter; rather, some of it is deposited on subsequently visited plants. The distance of gene migration is thus increased over the pollinator flight distances. Nonetheless, the pattern of gene migration within plant populations is limited (Fig. 1). Such experimental studies show restricted gene flow, but the specific pattern of gene flow may be modified by numerous factors, including seasonal variation in flowering (Schmidt, 1980), genotype (Tonsor, 1985), and the spatial distribution of plants (Handel, 1983; Ellstrand & Marshall, 1986). The most reliable estimates of gene flow come from paternity analysis within native populations (Ellstrand, 1984). Such studies

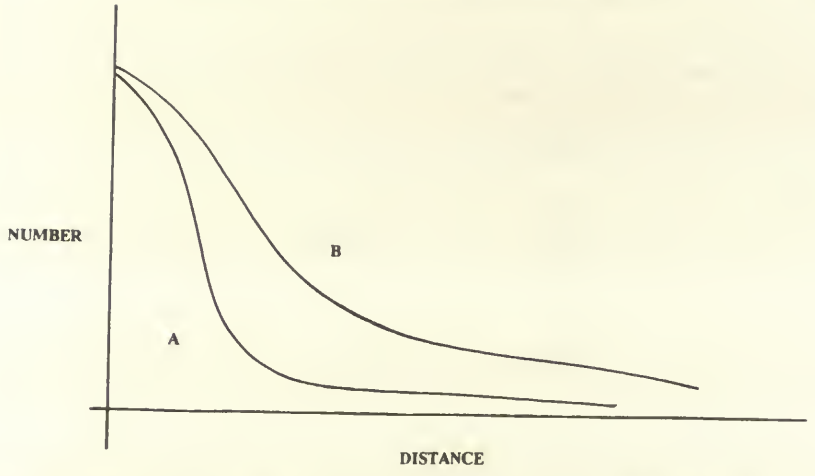

FIGURE 1. Pollinator moves and gene flow as a function of distance. A = flight distance of bees between plants. B = spatial movement of alleles. Based on data from Schaal (1980).

also show restricted gene flow but illustrate the mediating influences of spatial distribution and, potentially, selection in the pattern of gene migration (Marshall & Ellstrand, 1986).

III. Genetic Structure

Regardless of the exact pattern of gene migration, it is fairly conclusive that plant populations do not undergo random mating but that crossing distances are somewhat limited. In general, crossing relationships correspond to an isolation-by-distance model where the probability of crossing is a function of geographical distance between plants (Schaal, 1974). Sewall Wright has considered the consequences of such a pattern of mating, and several predictions on the genetic structure of plant populations arise from this work (Wright, 1946). On a theoretical basis, one expects that populations will be genetically subdivided if gene flow is limited. That is, populations will not consist of a random distribution of genotypes; rather, the population will show significant genetic heterogeneity of gene and genotype frequencies.

The spatial pattern of genetic variation has been analyzed in two populations of *Lupinus texensis* (Schaal, 1984a). Populations were divided into a series of grids, a sample of 20 plants within a quadrat was collected, and allozyme genotype was determined at three loci. The organization of spatial variation was analyzed by Sewall Wright's F-statistics (Table 2). F_{IS} measures the deviation of heterozygote frequencies from Hardy-Weinberg equilibrium within a quadrat. Deviation for Hardy-Weinberg equilibrium occurs in both populations for the three loci examined and averages 0.25. $F_{IS} = 0.0$ when the population is in Hardy-Weinberg equilibrium; $F_{IS} = 1.0$ when no heterozygotes occur in the population. Such a deviation from equilibrium is expected when either or both selfing and limited gene flow occur. Next the spatial distribution of gene frequencies was considered. F_{ST} measures the heterogeneity of gene frequencies within a population across sample quadrats. Both populations show significant genetic heterogeneity in gene frequency for the three loci studied, with an average F_{ST} of 0.16. Interestingly, the two populations differ slightly in the degree of local genetic differentiation within the populations. Population 1 is slightly more heterogeneous than is population 2. Such differences may reflect differences in local parameters of gene flow due to plant density, or they may reflect environmental heterogeneity within the population and selection at linked loci.

TABLE 2. Population subdivision in *Lupinus texensis* (from Schaal, 1984a). F_{IS} measures the deviation from Hardy-Weinberg equilibrium. F_{ST} indicates genetic heterogeneity among samples. F_{IT} is the total fixation index. See text for further explanation.

	locus	F_{IS}	F_{ST}	F_{IT}
population 1				
	ADH	0.28	0.19	0.41
	GOT	0.21	0.20	0.37
	6-PGDH	0.22	0.15	0.34
population 2				
	ADH	0.30	0.13	0.39
	GOT	0.28	0.14	0.38
	6-PGDH	0.20	0.17	0.34
mean		0.25	0.16	0.37

If the genetic heterogeneity within a population is the result of nonrandom mating due to pollinator foraging, then one expects the subpopulations to differ in gene frequency because of genetic drift. If such genetic differentiation occurs, the pattern of genetic differentiation should correspond to an isolation-by-distance model. Plants close to each other spatially will be similar genetically, since they will be consanguinous. As the geographical distance increases between plants the genetic distance will correspondingly increase. Thus, a second consequence of limited pollinator foraging is a correlation of genetic distance to geographical distance within a plant population. Such a realationship is illustrated in Figure 2, where, as geographical distance increases within a population, genetic distance also increases. Such a relationship between geographical and genetic distance has been found within a population of *Liatris cylindracea* (Schaal, 1974), and one would expect it to occur in other plant species such as *Lupinus texensis* where populations are subdivided due to restricted gene migration. An isolation-by-distance substructure within a population may influence the crossing relationships within the population. Price & Waser (1979) postulated differential success or fitness of offspring based on the geographical separation of parent plants within a population. Since plants in close proximity are consanguinous, the offspring produced by such a cross will be inbred and can potentially exhibit inbreeding depression. Moreover, plants located far apart in the population show little genetic similarity, and thus when crossed, two genetically disparate genomes combine and outbreeding depression may potentially occur. Figure 3 shows the fitness of offspring versus the geographical separation of parents within a population. Both the areas of inbreeding depression and outbreeding depression occur at the extremes of crossing distances. The region of optimal outcrossing occurs at intermediate distances and results in F_1 progeny that are neither inbred nor outbred and have maximum fitness. Price & Waser (1979) and others (e.g., Levin, 1984) have examined populations for such optimal outcrossing, and their data suggest that in some species such distance-dependent success in crossing may occur. Optimal outcrossing is a direct consequence of the pattern of pollinator foraging and the genetic substructure this imparts to the population. Thus the fitness of offspring is strongly influenced by pollinator behavior.

IV. Breeding System

Offspring fitness may be influenced much more directly by the interaction between plants and their pollinators. The pattern of pollinator foraging on an inflorescence will determine if selfing or outcrossing will occur. Since pollinators tend to move up the protandrous lupine flower from flowers which are predominantly female in function to flowers that are predominantly male in function, most crosses will be between plants, and on average little inbreeding will occur in the population. However, some moves are between inflorescences on the same plant, and in lupine

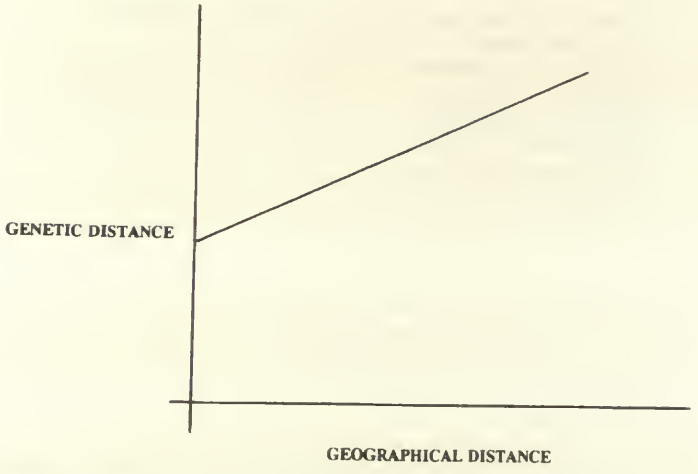

FIGURE 2. Isolation by distance within plant populations. Based on data from Schaal (1974).

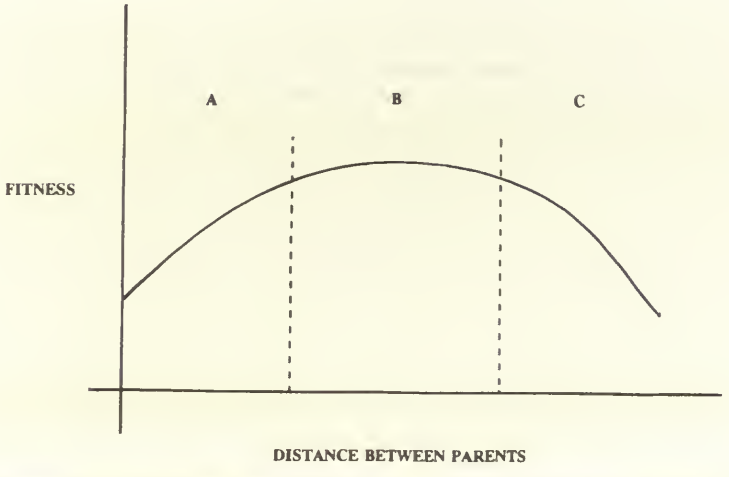

FIGURE 3. Optimal outcrossing within plant populations. A = region of inbreeding depression. B = outcrossing distance. C = region of outbreeding depression. Based on data from Price & Waser (1979).

populations we expect a low but significant level of selfing to occur. One expects the degree of selfing to vary among populations depending on the plant density, number of flowers and inflorescences per plant, and on the time of pollination during the flowering period. Furthermore, the consequences of selfing for the fitness of offspring should also vary among populations depending on the genetic load (the number of deleterious alleles held in the heterozygous state) of the populations. Inbreeding in populations with high genetic loads will lead to inbreeding depression, whereas selfing in populations with low genetic loads will result in relatively little inbreeding depression. In populations which routinely inbreed, one expects to see little or no fitness differences between inbred and outbred progeny.

We have examined the effect of breeding system (whether a plant is produced by selfing or outcrossing) on the fitness of plants (Schaal, 1984b). Before the influence of breeding system can

TABLE 3. Seed and seedling production effect of offspring seed size (from Schaal, 1984b).

% seed set vs. seed size	$r = -0.25$
Seeds/fruit vs. seed size	$r = -0.36$
% germination vs. seed size	$r = 0.49^*$
Time of germination vs. seed size	$r_s = 0.61^*$

$^*p < 0.05$

be determined, the influence of maternal effects on the fitness of progeny must be considered. The maternal or seed parent influences the fitness of its offspring in many ways. Genetically it determines one half of the genome and all of the chloroplast genome in most cases. The seed parent is responsible for many seed characteristics, including those that influence dispersal. Maternal effects, as considered here, are a result of the nutritive relationship that the seed-producing parent has to its developing embryo. In plants such as *Lupinus*, the developing seed is directly dependent on the maternal plant for resources. The size of the seed is often related to how well a maternal plant fulfills its maternal role in supplying the developing embryo with resources. Seed size, in turn, can be of overwhelming importance in determining the fitness of progeny and can obscure or mediate the influences of the embryo genotype and hence breeding system.

We begin our discussion of breeding system and offspring fitness by considering maternal influences on fitness. The study below examines both the influence of maternal effects and breeding system simultaneously in greenhouse populations of *Lupinus texensis* (Schaal, 1984b). Plants were grown in the greenhouse from field-collected seed. Nineteen plants were repeatedly crossed with both self-pollen and with a mixed, outcross pollen load. The fitness of the resulting progeny were examined by a detailed life history analysis. The success of self-crosses versus outcrosses was measured by the percent seed set, seed weight, and the number and frequency of aborted seeds. The resulting seeds were of variable weight and produced either by inbreeding or outbreeding. Seeds were planted in the greenhouse, and the resulting plants were followed throughout their life spans. Plants were scored weekly for growth until eight weeks subsequent to germination, and then survivorship and fecundity were scored weekly until the plants died.

The relationship between seed size and early components of fitness is shown in Tables 3 and 4. Seed size is not correlated with seed set or seeds per fruit. Thus, the production of seeds is not influenced by their size. Once seeds are produced, seed size then has a strong influence on fitness. Both the percentage germination and the timing of germination are correlated to seed size; larger seeds germinate early and have an increased percentage of germination (Table 3). The size of plants after germination is shown in Table 4. Plant size is positively correlated to seed size up to the fourth week after germination; after this, the correlation between seed size and plant size is no longer significant. Table 5 shows the age-specific survivorship values for the plant life cycle correlated to seed size. Seed size again shows a relationship to fitness; seed size is correlated to survivorship early in the life cycle but is no longer significant after the fourth week subsequent to germination. The average amount of reproduction per individual, the net reproductive rate, shows a nonsignificant correlation to seed size, ($r = 0.36$, $P > 0.05$). But if reproduction is broken down into age-specific components, reproduction at the most intense time during the flowering period is significantly correlated to seed size (Table 6). This correlation is probably a secondary effect. Large seed produce large seedlings; large seedlings in turn produce large plants, which reproduce more. It can be concluded from these studies that maternal effects are of predominant importance early in the life cycle of a plant and wane after the fourth week following germination. The period of time when maternal effects are strong can be of long duration and is an important time in the plant life cycle. The period between seed production and one month after germination encompasses both the time an individual spends in the seed pool, which may be years, and the time of seedling establishment, which is considered a critical period for survival in the life cycle of a plant.

TABLE 4. Seed size vs. germination and plant size (based on data from Schaal, 1984b).

	Germination Time	Number of Leaves 2 weeks	Number of Leaves 4 weeks	Number of Leaves 6 weeks	Number of Leaves 8 weeks
r	0.05	0.53	0.54	0.28	0.22
Number of pops (25)	9	15	13	6	3

TABLE 5. Survivorship (1_x) vs. seed size (from Schaal, 1984b).

Time (weeks)	2	4	6	10	14	18	22	24	26	28	life span
r	0.56*	0.45*	0.20	0.18	0.18	0.18	0.17	−0.06	−0.17	−0.37	0.10

*p <0.05

Next, we consider the effect of plant genotype on fitness. The movement of pollinators within a plant population will determine whether seeds are produced by self-fertilization or cross-pollination. Here we examine the consequences of offspring fitness for selfing and outcrossing. The effects of progeny genotype, whether inbred or outbred, on seed and seedling production is shown in Table 6. In this population there is no effect of embryo genotype on any measure of fitness early in the life cycle. Selfing or outcrossing does not influence seed set, seeds per fruit, seed abortion, weight, or germination (Table 7). Plant growth after germination is examined in Table 8. Early in the life

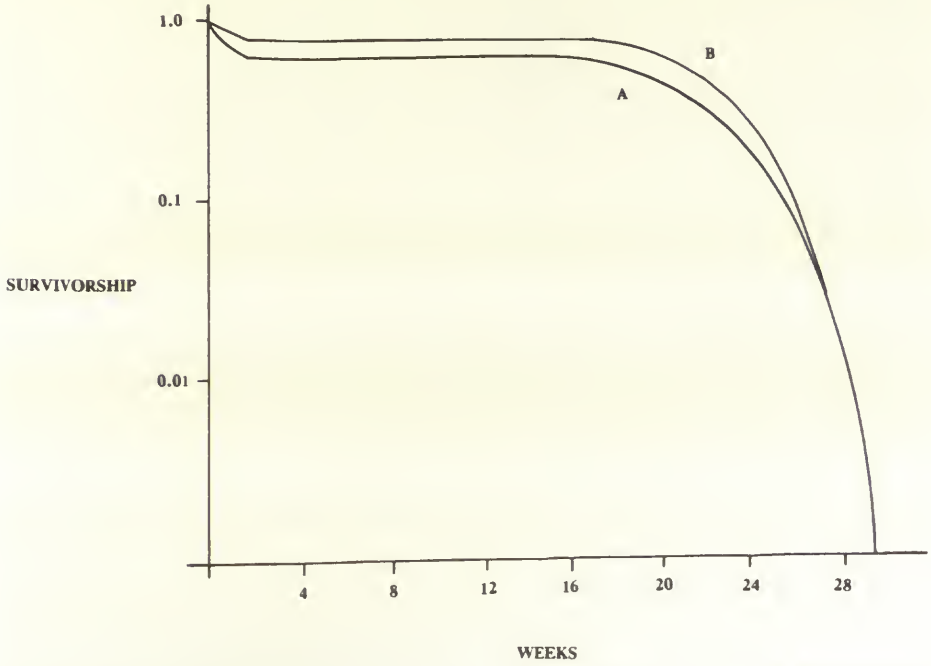

FIGURE 4. Survivorship of selfed and outcrossed progeny. A is selfed; B is outcrossed. Based on data in Schaal (1984b).

TABLE 6. Age specific reproduction (m_x) effect of seed size (from Schaal, 1984b).

Age class	m_4	m_5	m_6	m_7	m_8	m_9	m_{10}	m_{11}	m_{12}	m_{13}	m_{14}
r	0.30	0.16	0.34	0.56*	0.56*	0.62*	0.49*	0.34	0.23	0.10	0.21

*p <0.05

TABLE 7. Seed and seedling production, selfed vs. outcrossed progeny (from Schaal, 1984b).

	Self	Outcross
% seed set	12.15 (n = 2,708)	11.46* (n = 1,571)
Seeds/fruit	4.57 (n = 214)	4.56* (n = 104)
Viable seeds/fruit	2.86	3.08*
Aborted seeds/fruit	1.71	1.48*
Seed weight	38.65 (n = 206)	39.58* (n = 223)
% germination	91.55 (n = 206)	91.03* (n = 223)

*p <0.05

TABLE 8. Plant size, selfed vs. outcrossed progeny (from Schaal, 1984b).

	Self	Outcross	t
2 weeks			
Number of leaves	2.62 (n = 167)	2.67 (n = 192)	0.317
4 weeks			
Number of leaves	8.94 (n = 154)	10.76 (n = 176)	4.12*
6 weeks			
Number of leaves	20.08 (n = 144)	23.44 (n = 172)	2.14*
8 weeks			
Number of stems	5.22 (n = 140)	5.56 (n = 166)	1.99*

*p <0.05

cycle there is no detectable influence of breeding system on the fitness of progeny. Self and outcross progeny show no difference in size until the fourth week after germination. After the fourth week there are significant differences in offspring fitness between inbred progeny and outcrossed progeny. Four weeks after germination, selfed plants are smaller and continue to be smaller (Table 8). Survivorship of inbred plants is lower (Fig. 4) so that the mean life span of inbred plants is 140 days versus 165 days for outbred individuals. Levels of reproduction also differ between inbred and outbred progeny (Fig. 5), with outbred plants having a much higher reproductive rate than do inbred plants. The mean number of flowers for outbred plants is 107.8 versus 88.4 for the progeny of self crosses.

This study shows that the consequences of pollinator behavior in producing selfed versus outcrossed seed strongly influence the fitness of progeny. Individuals that outcross are expected to produce more fit progeny, all other things being equal, than plants that inbreed. This relationship to fitness is mediated by maternal effects. Maternal effects are of predominant importance in determining fitness early in the life cycle of the plant. Only when the influence of the maternal effect wanes are their differences in fitness associated with progeny genotype.

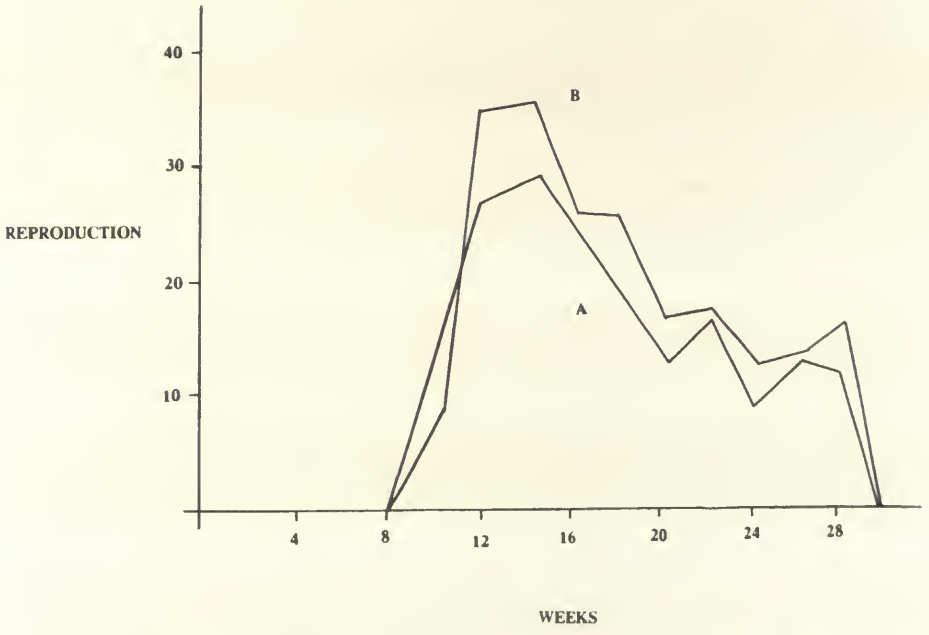

FIGURE 5. Reproduction of selfed and outcrossed progeny. A is selfed; B is outcrossed. Based on data in Schaal (1984b).

V. CONCLUSIONS

The association of a legume flower and its pollinator has major consequences for the plant which extend far beyond obtaining a reliable source of pollination. The pattern of pollinator foraging influences the genetic structure of a species, the degree of local differentiation within populations, and the genetic differences among populations. Pollinator foraging will also influence the pattern of mating among and within plants in the population and thus influence plant genotype by producing selfed or outcrossed progeny. Plant genotype, in turn, strongly influences the fitness of the plant. Thus both the genetic structure of plant populations and the fitness relationships among individuals are strongly affected by the pattern of pollinator foraging among the plants of a population.

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REFERENCES

ARROYO, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

BABBEL, G. R. & R. K. SELANDER. 1974. Genetic variability in edaphically restricted and widespread plant species. Evolution 28: 619–630.

Ellstrand, N. C. 1984. Multiple paternity within the fruits of the wild radish, *Raphinus sativus*. Amer. Naturalist 123: 819–823.

_____& D. L. Marshall. 1986. Patterns of multiple paternity in populations of *Raphanus sativus*. Evolution 40: 837–842.

Ennos, R. A. & M. T. Clegg. 1983. Effect of population substructuring on estimates of outcrossing rate in plant populations. Heredity 48: 282–292.

Handel, S. 1983. Contrasting gene flow patterns and genetic subdivision in adjacent populations of *Cucumis sativis*. Evolution 37: 760–771.

Harper, J. 1977. The Population Biology of Plants. Academic Press, London.

Levin, D. A. 1981. Dispersal versus gene flow in plants. Ann Missouri Bot. Gard. 68: 233–253.

_____. 1984. Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. Evolution 38: 116–127.

Marshall, D. L. & N. C. Ellstrand. 1986. Sexual selection in *Raphanus sativus:* experimental data on non-random fertilization, maternal choice, and consequences of multiple paternity. Amer. Naturalist 127: 446–461.

Price, M. V. & N. M. Waser. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature 277: 294–296.

Schaal, B. A. 1974. Isolation by distance in *Liatris cylindracea*. Nature 252: 703.

_____. 1980. Measurement of gene flow in *Lupinus texensis*. Nature 284: 450–451.

_____. 1984a. The comparative population biology of two lupine species. Pp. 439–452 *in* W. J. Grant (editor), Biosystematics. Academic Press, Toronto.

_____. 1984b. Life-history variation, natural selection, and maternal effects in plant populations. Pp. 188–206 *in* R. Dirzo & J. Sarukhán (editors), Perspectives on Plant Population Ecology. Sinaur Assoc., Sunderland, Massachusetts.

_____& W. J. Leverich. 1980. Pollination and banner markings in *Lupinus texensis*. Southw. Naturalist 25: 280–282.

Schmitt, J. 1980. Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae). Evolution 34: 934–943.

Tonsor, S. 1985. Intrapopulational variation in pollen-mediated gene flow in *Plantago lanceolata*. Evolution 39: 775–782.

Wright, S. 1946. Isolation by distance under diverse systems of mating. Genetics 31: 39–59.

NATURAL HISTORY OF A WIND-POLLINATED CENTRAL AMERICAN DRY FOREST LEGUME TREE (*ATELEIA HERBERT-SMITHII* PITTIER)

Daniel H. Janzen[1]

Abstract

Ateleia herbert-smithii Pittier (Papilionoideae) is a moderate-sized dioecious tree endemic to about 80 km^2 of Santa Rosa National Park (250–350 m elevation) within the widespread dry forest of northwestern Costa Rica, and to an equally small area of severely perturbed dry forest in central western Nicaragua. This paper summarizes 11 years of close observation of this tree in Santa Rosa. *A. herbert-smithii* is unambiguously wind-pollinated (although the male trees are heavily visited by pollen-collecting social bees), and is the only known wind-pollinated legume in the world (however, other members of the genus are probably also wind-pollinated). Males produce pollen in pulses, and the females are continuously receptive for several weeks. Flowering and fruiting was synchronized within the population at two-year intervals from 1974 to 1984, but this perfect synchrony was disrupted by an odd-year and population-wide flowering in 1985. A large adult *A. herbert-smithii* may produce as many as a million one-seeded fruits in a crop, but more than 90% of the seeds in most crops are killed by the larvae of a single species of a monophagous weevil (Curculionidae: *Apion johnschmitti*) while the fruits are green and the seeds are just developing. In most cases, it appears that the weevils are sufficiently efficient that seed predator satiation does not occur even in the largest seed crops; instead most seed escape appears to be due to various kinds of "errors," such as when there are two seeds in a fruit and the weevil larva eats only one. The mature dormant seeds are rejected as food by the common generalist seed predator in Santa Rosa, the spiny pocket mouse (Heteromyidae: *Liomys salvini*). The foliage of *A. herbert-smithii* is eaten by very few species of insects and not browsed by vertebrates; it is relatively free of phenolics but very rich in aromatic compounds. The wind-dispersed fruits move the seeds up to several hundred meters into open pastures. If the pasture is fire-free, *A. herbert-smithii* is an aggressive colonizer and shades out the grass. While the large adults persist in secondary succession for up to 100–200 years, recruitment does not occur in forest shade and the species gradually disappears except in oak forest and a few adjacent deciduous forest types. The very small geographic distribution of this tree appears to be due to a combination of its dioecious behavior, its wind-pollination, its wind-dispersal, and its slow rate, for a tree of early successional stages, of reaching an age of abundant seed production.

I. Introduction

Most species of lowland Mesoamerican trees have (had) broad geographic distributions, are pollinated by animals, and suffer zero to moderate predispersal seed predation. *Ateleia herbert-smithii* Pittier, a moderate-sized papilionoid legume tree (Figs. 1, 2), is a strong exception to all three generalizations. Here I describe the natural history of this tree in Santa Rosa National Park in northwestern lowland Costa Rica (35 km north of Liberia in Guanacaste Province, 0–350 m elevation, 10°50′N, 85°40′W); the park is occupied by one of the two known surviving *A. herbert-smithii* micropopulations. The natural history of this tree is of more than esoteric interest. It has the potential of becoming a widely introduced tropical fuelwood tree (Hughes & Styles, 1984, and this volume) and may also be introduced as a woody rapid colonizer in severely deforested habitats; its natural history deserves scrutiny as background for management and weed potential.

Its common name in Nicaragua is "palo prieto" (C. Hughes, B. T. Styles, R. Lopez, L. D. Gomez, pers. comm.), but in the Santa Rosa area it has no vulgar epithet. It is, however, sometimes

[1]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.

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FIGURE 1. Crowns of two adult male *Ateleia herbert-smithii*. The upper tree is 15 m tall and the lower is 20 m tall. Both trees are in full leaf (August). They have more major and minor branches, and the branches are longer and thinner than is the case with females of the same age and exposure to sun. Santa Rosa National Park, Guanacaste Province, Costa Rica.

FIGURE 2. Crowns of two adult female *Ateleia herbert-smithii*. The upper tree is 10 m tall and the lower is 21 m tall (note 1.5 m tall person in crown for scale). Both trees are bearing heavy fruit crops in January; the upper tree is leafless and the lower tree has shed most of its leaves. Same locale as Figure 1.

given the name of "chaperno," a general term for *Lonchocarpus* species (Papilionoideae) in the Santa Rosa area. As it becomes the focus of biological interest, it is becoming known in the park as "ateleia" in both English and Spanish.

II. Geographic and Ecological Distribution

Ateleia herbert-smithii was described in 1918 from an 18–m tall tree growing "5 miles south of Mamatoco, Magdalena Province, Colombia" (also recorded in flower "3 miles north of Bonda"), and Velva Rudd commented in 1968 (1968: 405) that "this distinctive species has never been re-collected. Two Colombian botanists, Dr. Armando Dugand and Dr. R. Romero-Castañeda, have unsuccessfully searched for living specimens. It may be another rare endemic that has succumbed to human activity." While it may be subsequently relocated as a small population or population fragment in Colombia, it is clearly not a widespread or common tree in that country. In 1975, Rudd identified specimens from the population of *Ateleia* growing in Santa Rosa National Park as *A. herbert-smithii* (pers. comm.), and herbarium specimens from that population have been deposited in the U.S. National Herbarium and the Missouri Botanical Garden (and then distributed from there as well). In 1982, Colin Hughes and Brian Styles discovered a small and highly disturbed population of *A. herbert-smithii* growing in the neighborhood of the village of El Bordo las Tinajas, about 2 km southeast of La Cruz de la India, on the road from Sebaco to Telica and Leon, Leon Department, Nicaragua (350–450 m elevation)(pers. comm.). In 1976, a specimen of *A. herbert-smithii* was collected 10 km north of Ciudad Dario (500 m; *D. Neill 1249*); Colin Hughes and Douglas Stevens are of the opinion that this collection is from the same population as grows at the El Bordo site (pers. comm.). Finally, the Missouri Botanical Garden has a 1983 collection of what appears to be *A. herbert-smithii* from north of Puerto Cabezas, Tuapi, Zelaya Department, Nicaragua (15 m; *J. C. Sandino 4039*; Flora of Nicaragua project). However, this site is much wetter and bears a very different vegetation from that occupying the other sites where *A. herbert-smithii* occurs; this suggests that the specimen may be misidentified.

The very patchy and local distribution of *Ateleia herbert-smithii* implied by the above records is probably an accurate statement about the pre- and post-Spanish distribution of the tree for the following reasons: 1) Plant collecting has been intensive in Mesoamerica, especially during the past 20 years for a variety of local and regional floras. The absence of *A. herbert-smithii* in collections probably means that it is absent in the field, although of course there still remains the possibility that some tiny population has been overlooked. 2) *Ateleia herbert-smithii* is a moderately large and conspicuous tree, and therefore it is unlikely to be missed by collectors. 3) *Ateleia herbert-smithii* is a tree of dry and intermediate elevation sites, and such sites have maximal accessibility to collectors. 4) Most of the Mesoamerican dry forest has been thoroughly agriculturalized. However, this habitat is still overlain with a filmy net of secondary successional fragments (fencerows, woodlots, secondary succession following pasturing and lumbering) that would harbor old adults and some juveniles of *A. herbert-smithii* as remnants of widespread populations if such widespread populations had been present when the European agriculturization of Mesoamerica began in the late 1500s. The ruderal behavior of this species (see below) reinforces this conclusion. However, its reliance on wind-pollination and its high seed mortality suggest a species that is easily extinguished if its density declines. If it originally occurred only as micropopulations on particular sites, many of these could have been obliterated by agriculturization (as Rudd suggested for the Colombian population). The latter two aspects of its biology mean that its overall rareness may be a human-generated artifact.

There are about 17 known species of *Ateleia* (all neotropical and most Mesoamerican [Rudd, 1968; Dillon, 1980]) and "all species are known from limited ranges with the exception of *A. gummifera*, which ranges throughout the West Indies and eastern coast of Central America"

(Dillon, 1980: 551). They all have similar flowers and fruits, and I suspect that all have (had) biologies quite similar to the one described below for *A. herbert-smithii* at Santa Rosa. *Ateleia herbert-smithii* is the only member of the genus that has been examined in detail in the field, in the greenhouse (C. Stirton, C. Hughes, and B. Styles), and in the laboratory (S. Tucker, this volume). I suspect that the ecological and geographic traits described here for *A. herbert-smithii* are (were) representative of most species of the genus except for *A. gummifera*.

The trees of Costa Rica have been very thoroughly collected over the past 100 years, many field biologists have worked throughout the country at least as long, and I have conducted field work for 25 years on the dry side of Costa Rica (e.g., Janzen, 1980). All of this collecting and observing has located one specimen of *Ateleia gummifera* (where the Río Higueron crosses the Interamerican Highway about 10 km south of Cañas in Guanacaste Province; S. Salas, pers. comm.) and the Santa Rosa population of *A. herbert-smithii* (which I first sampled in 1965 but could not identify at that time). I have been unable to locate any living plants of *A. gummifera* in the heavily agriculturized area where it was once collected (or elsewhere) and conclude that it has probably been recently extinguished in Costa Rica.

The detailed distribution of *Ateleia herbert-smithii* at Santa Rosa was determined by careful exploration on foot and in off-the-road vehicles, beginning within the obvious population lying along the 6 km of asphalt highway between the entrance of Santa Rosa National Park on the Interamerican Highway (35 km north of Liberia) and the park administration area, and then exploring outward until no more plants could be found. This method does not, however, census dormant seeds in the soil, seeds that may still be present in the 1–8-year-old newly cleared fields of Hacienda Ahogados (Finca Guapote) and Hacienda Rosa Maria along the southern boundary of Santa Rosa. The *A. herbert-smithii* population occupies approximately 80 km^2. It occurs in the area that is roughly bounded by the following locally well-known landmarks: the northern boundary of Hacienda Rosa Maria at its airstrip, to the Interamerican Highway, to about 1 km south of the entrance of Hacienda Poco Sol, to the Laguna Escondida in Santa Rosa, to 1 km west of the intersection of Quebrada Cebollines with the road to Playa Narango, to Quebrada Las Dantas, and thence back to the Rosa Maria airstrip. All of this area is about 250–350 m elevation.

Needless to say, if Santa Rosa National Park had been established only 20 km away along any compass direction, the population would either be as badly altered as is the El Bordo population in Nicaragua, or extinct, depending on the agricultural practices on the site. Santa Rosa has been a low-grade cattle ranch for over 400 years; that the population survived long enough to be incorporated into a national park is mostly due to the relatively low quality of the site for ranching and agriculture (Janzen, 1988); the long distance of the site from the population and political centers of Managua, Nicaragua, and San José, Costa Rica; and the ability of the plant to colonize and persist in dry forest successional microhabitats that are not burned annually.

Except for some *A. gummifera* records, virtually all *Ateleia* collection sites (Rudd, 1968; Dillon, 1980) are from Mesoamerican sites with a 5–7 month relatively rain-free dry season and a well-developed rainy season (1–2 m rainfall). South of the Isthmus of Tehuantepec in southern Mexico, these sites are often occupied by lowland oaks (e.g., *Quercus oleoides*) and edges of brushy or abandoned pastures. However, the microdistribution of *A. herbert-smithii* within Santa Rosa shows clearly that at least *A. herbert-smithii* does not require *Quercus* as an associate; only about half of its distribution in Santa Rosa presently co-occurs with *Q. oleoides* (but see the discussion for the possibility that the original ecological distribution of *A. herbert-smithii* was much narrower than at present). The forested habitats that *A. herbert-smithii* occupies tend to be at the dry end of the Mesoamerican climatic spectrum. Like most other Mesoamerican dry forest habitats, these areas are exceptionally prone to pasturization by partial forest cutting followed by cattle and annual fires (e.g., see Uhl & Buschbacher, 1985). *A. herbert-smithii* juveniles are exceptionally fire-sensitive and therefore they do not recruit well in Costa Rican dry forest pasture habitats, even though they are not browsed by cattle.

At Santa Rosa, *Ateleia herbert-smithii* is restricted to an elevation of about 250–350 m, but it

does not occupy all terrain of this elevation even when this terrain extends for many kilometers away from the park.

Where there are adult seed trees at Santa Rosa, *Ateleia herbert-smithii* individuals of various ages are obvious and common members of unburned woody primary and secondary succession along roadsides, in abandoned and unburned pastures, in tree falls, in fencerows, in very deciduous forest understory (although the flimsy seedlings and saplings do not survive unless a gap occurs over them), and on landslides and cliff edges. The species grows on soils ranging from nearly bare rock on the tops of welded ash flows to pockets of deep volcanic alluvium of high quality for agriculture. Adult *A. herbert-smithii* occur from water-logged and swampy black clay soils to well-drained rock outcrops; over this distribution, however, the tree is much more common on the dry than the wet end of the gradient. It gives the impression of a very robust tree, successful in meeting the challenges of a highly seasonal tropical climate, poor soils, multi-species competition, and high herbivore loads. Although *A. herbert-smithii* is not eaten by horses and cattle, a fire hot enough to kill the aboveground parts kills the entire tree, sapling, or seedling. It is not shade tolerant. Given these traits, it is not at all obvious why *A. herbert-smithii* is not a widespread member of secondary succession throughout Pacific Central America. Its only obvious detrimental traits are its sensitivity to fire, the need for insolation for the seedlings and saplings, and its slowness to reach maturity. It does not begin to produce large fruit crops until it is at least 20–30 years old.

III. Reproduction

Overview

Ateleia herbert-smithii seedlings appear immediately after the first heavy rains in late April or May and sprout from dormant hard seeds that may range in age from that year's fruit crop to three or more years old. Dormancy ends by the seed coat being broken (scarified) by drying, fungal or microbial etching, soil abrasion, etc. However, many seeds require no treatment other than placement on moist soil in order to germinate. Saplings of both sexes, growing in full sun, produce their first flowers at about 5–6 years of age and 3–4 m height. A large fruit crop may appear on a 15-year-old tree that is 4–6 m in height, but 20–30 years is a more normal age for *A. herbert-smithii* to begin to bear large fruit crops. The tree continues to flower at approximately biennial intervals for at least 100 years. Adults are dioecious (Figs. 1–5) and retain their sexual category throughout life. A sample of 50–100 adults chosen randomly always has a 50:50 sex ratio. Most flowering occurs in mid October through mid November, but some individuals flower as much as one month later or longer. The tiny white to greenish flowers (Figs. 3–5) are borne in multi-flowered elongate inflorescences, and the opening progression is from base to tip. Females continually open new flowers on an inflorescence over a period of 2–3 weeks, and an individual plant's flowers may open over a 2–5 week period. Most of the male flowers on a tree open 1–3 pulses of one morning's duration each, and individual male plants are synchronized to flower within a 3–5 week period. Pollen is heavily harvested by social bees, but pollen flow to female trees is entirely by wind. The size of fruit crops is determined in part by the favorableness of the weather for wind-mediated pollen flow. Crops range from a few tens or no fruits to two million per year (Table 1; Appendix I). The 1- or 2-seeded fruits (Figs. 6, 7) begin to expand within a few days of pollination, and seemingly full-sized immature seeds are on the tree within 1–2 months after pollination. The fruits begin to dry in the second month of the dry season (January). In February–April they are dry enough to be blown from the tree, although there is day-to-day variation due to the strength of the wind and heat of the day. The seeds in about 90% of the fruits are killed by the developing larvae of *Apion johnschmitti* Whitehead (Whitehead, 1985), a curculionid weevil, before fruit dispersal occurs (Fig. 6; Appendices I, V–VII; Tables 2–4, 6, 7).

The tree flowers near the end of one year and disperses its mature fruits in the early part of the

FIGURE 3. Inflorescences of male (upper) and female (lower) *Ateleia herbert-smithii* in full flower. The male flowers opened the morning of the photograph, while the female inflorescences bear full-sized green fruits at their bases and unopened buds at their tips. Same locale as Figure 1.

FIGURE 4. Male inflorescence (upper), flowers (middle), and pollen thief (bottom: *Trigona fulviventris*) of *Ateleia herbert-smithii* on the morning of opening. Same locale as Figure 1.

FIGURE 5. Female inflorescences of *Ateleia herbert-smithii*. On the right, the uppermost flower has just opened on the day of the photograph. Same locale as Figure 1.

TABLE 1. Estimated size of fruit crops per tree for *Ateleia herbert-smithii* in Santa Rosa National Park, 1974–1985.

	1974	1976	1978	1980	1982	1984	1985
Average	89,750	42,882	141,298	114,196	177,797	37,420	48,421
s.d.	51,796	63,354	218,373	133,278	278,798	82,851	140,641
n	20	33	18	25	101	37	60
Maximum	200,000	270,079	646,700	426,136	1,944,000	444,814	922,208
Minimum	20,000	944	10	1,100	124	38	762
Range	180,000	269,135	646,690	426,125	1,943,876	444,776	921,446
Median	75,000	18,672	23,106	53,952	66,302	9,300	11,927
Skewness	0.79	1.96	1.37	1.15	3.55	3.51	4.9
Kurtosis	−0.38	3.25	0.33	0.09	16.47	13.35	25.03
Probability distribution normal	0.221	0.034	0.067	0.164	0.004	0.019	0.002

next year; I have adopted the convention of using the flowering year as the label for a flowering-fruiting event (the alternative convention of using the fruiting year was unfortunately followed in Janzen, 1978).

SEEDS

The mature and living seeds of *Ateleia herbert-smithii* are flattened ovoids, very hard, smooth, red-brown, and 5–6 mm long by 3–4 mm wide. The seed coat is 0.08–0.15 mm thick and

FIGURE 6. Infructescences and individual fruits of *Ateleia herbert-smithii*. The infructescences at top are about as fruit-rich as occur, with as many as 700 fruits on the largest ones. In the lower figure, the ruler is in mm, the exit hole was made by an adult *Apion johnschmitti* weevil, and several dead weevils have been placed next to a seed (exposed by dissection), the contents of which were eaten by a weevil larva. The intact seed exposed by dissection is full-sized but not mature. Same locale as Figure 1.

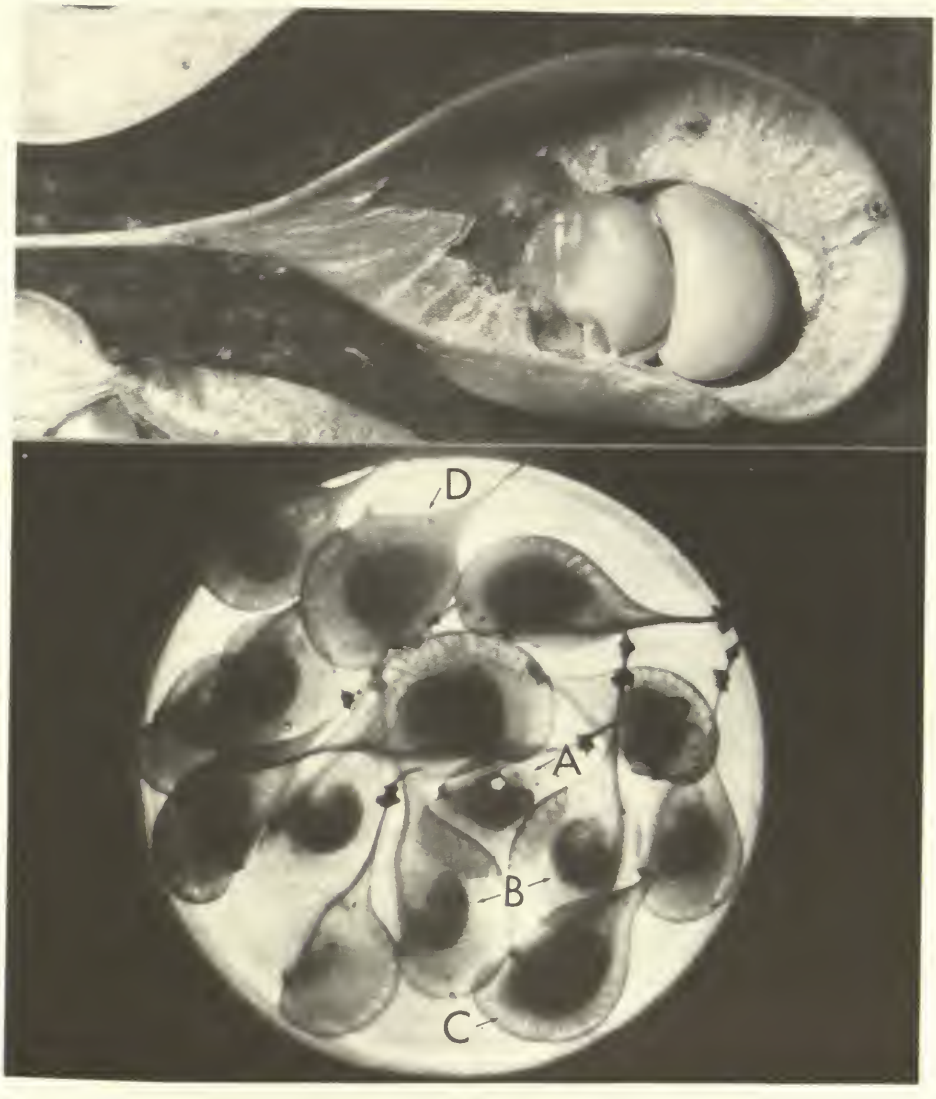


FIGURE 7. Dissected 2-seeded fruit of *Ateleia herbert-smithii* (upper). "Candled" fruits viewed by transmitted light (lower). The fruit labeled A has a weevil exit hole, and those labeled B are two other fruits with weevil-killed seeds. Fruit C has an intact seed. Fruit D has two seeds, one of which has been killed by the weevil. Same locale as Figure 1.

surrounds a layer of translucent hard and polysaccharide-rich endosperm, which in turn surrounds the cream-white and hard embryo. In the green developing fruit, the embryo is dark green and packaged in a clear endosperm and a white translucent seed coat (Fig. 6). (I assume that the green embryo is photosynthetic, cf. Janzen, 1982a.) Living, dormant, and dry *A. herbert-smithii* seeds are about 40% seed coat by live mass; and when the seed is oven-dried, the seed coat is 43% of the dry mass of the dead seed (Janzen, 1978). This approximate ratio of 3:2 seed contents to inert packaging

TABLE 2. Number of *Ateleia herbert-smithii* seeds that escaped from *Apion johnschmitti*, per tree, from 1974 to 1985 in Santa Rosa National Park.

	1974	1976	1978	1980	1982	1984	1985
Average	44,915	5,856	50,486	10,391	5,769	2,344	1,551
s.d.	34,134	12,271	110,228	17,245	8,442	6,337	4,228
n	20	33	18	25	101	37	60
Maximum	142,675	68,293	397,080	76,418	47,368	34,443	26,565
Minimum	4,365	28	10	0	20	8	12
Range	138,310	68,265	397,070	76,418	47,348	34,435	26,553
Median	42,730	1,460	4,433	2,864	2,590	183	234
Skewness	1.16	4.01	2.2	2.43	2.45	3.9	4.5
Kurtosis	1.13	17.39	3.56	6.08	6.48	15.8	21.38
Probability distribution normal	0.167	0.034	0.047	0.086	0.006	0.015	0.003
% total in largest crop	16	35	44	29	8	40	29
Tree with largest crop	18	177	UM	177	285	294	UM
% total in 2 largest crops	27	45	75	45	14	60	48

TABLE 3. Percent *Ateleia herbert-smithii* fruits containing seeds attacked by *Apion johnschmitti*, per tree, 1974–1985 in Santa Rosa National Park.

	1974	1976	1978	1980	1982	1984	1985
Average	61.55	84.68	68.23	85.54	95.1	92.04	97.99
s.d.	19.2	14.08	27.99	26.42	10.22	12.14	1.01
n	20	33	18	25	101	37	60
Maximum	89.63	99.38	96.95	97.7	99.47	99.6	99.35
Minimum	23.64	49.76	0	0	25.17	41.73	92.92
Range	65.99	49.62	96.95	97.7	74.73	57.87	6.43
Median	64.17	87.41	76.09	91.43	97.36	95.99	98.19
Skewness	−0.25	−0.84	−0.93	−2.5	−5.69	−2.66	−2.59
Kurtosis	−1.2	−0.34	−0.18	5.05	34.09	6.95	9.54
Probability distribution normal	0.264	0.197	0.21	0.038	0.5	0.025	0.117

TABLE 4. Percent seeds attacked by *Apion johnschmitti*, per tree, 1974–1985 in Santa Rosa National Park.

	1974	1976	1978	1980	1982	1984	1985
Average	56.15	82.59	68.23	82.82	94.16	91.55	97.03
s.d.	19.08	14.6	27.99	26.35	10.42	12.49	1.81
n	20	33	18	25	101	37	60
Maximum	89.63	99.38	96.95	97.56	99.47	99.6	99.35
Minimum	23.64	49.76	0	0	24.96	41.73	89.97
Range	65.99	49.62	96.95	97.56	74.51	57.87	9.38
Median	52.95	86.05	76.09	90.96	96.91	95.74	97.5
Skewness	0.22	−0.49	−0.93	−2.45	−5.22	−2.48	−1.65
Kurtosis	−1.16	−0.95	−0.18	4.86	29.92	5.82	2.93
Probability distribution normal	0.287	0.182	0.21	0.055	0.001	0.028	0.086

material was maintained over a 1.9-fold range of seed masses. After they have been scarified, healthy-appearing seeds display 100% germination within a few days when placed in moist soil, and a large number of seeds germinate directly without being explicitly scarified. Seeds that have been kept dry for six years will germinate when scarified and placed in moist soil; however, I doubt that

TABLE 5. Percent *Ateleia herbert-smithii* fruits with two seeds in them, per tree, 1974–1985 in Santa Rosa National Park.

	1974	1976	1978	1980	1982	1984	1985
Average	11.35	2.45	not available	1.44	1.26	0.8	2.23
s.d.	18.98	5.3		3.69	3.15	2.86	4.21
n	20	33		25	101	37	60
Maximum	50	19		14	20	17	21
Minimum	0	0		0	0	0	0
Range	50	14		14	20	17	21
Median	0	0		0	0	0.1	1
Skewness	1.11	2.05		2.78	3.95	5.3	3.06
Kurtosis	−0.66	2.9		6.27	17.27	27.87	9.12
Probability distribution normal	0.047	0.01		0.026	0.5	0.009	0.006

A. herbert-smithii seeds retain their dormancy through more than 2–3 rainy seasons in the field, and the bulk of the seeds probably germinate in the first rainy season following seedfall.

The mean seed mass of dry living seeds from single-seeded fruits on 18 trees in the 1974 fruit crop ranged from 32 to 60 mg, and viable seeds (from 2-seeded as well as 1-seeded fruits) ranged from 20 to 76 mg in mass (Janzen, 1978; note that the numbers assigned to seed crops in Janzen, 1978, do not correspond to the actual tree identification numbers used in Appendix I in the present paper). Different trees had significantly different mean seed masses (1.89-fold range in means), and trees ranged from having almost all of their seeds with a mass less than that of the grand mean (48 mg) to almost all seeds being heavier than the grand mean (fig. 2 in Janzen, 1978). Within a seed crop there was a 1.6- to 2.6-fold range in individual seed masses, and this broad distribution was maintained in the presence of a 4-fold variation in possible seed masses over all seed crops. In other words, as a tree's average seed mass increases, all the seeds increase about the same amount in mass.

Seeds from 1-seeded fruits had significantly more mass than did seeds from 2-seeded fruits (Janzen, 1978). However, the combined weight of an average pair of seeds in a 2-seeded fruit had more mass than did the average seed from a 1-seeded fruit. In a large sample of viable seeds pooled from 13 Nicaraguan trees at the El Bordo Las Tinajas site, there were 17,000 viable seeds/kg (C. Hughes, pers. comm.).

The percent of 2-seeded fruits (Fig. 7) varied from none to as high as 50% of the seed crop, but was most commonly from about 0–20% of the fruit crop (Fig. 8e, f; Table 5; Appendices I, V–VII). I have found only two fruits with three seeds in them and none with a greater number in the tens of thousands of fruits examined in this study.

Whole ground seeds (and leaves) of *Ateleia herbert-smithii* from Santa Rosa contained 1–3% dry mass of three uncommon amino acids (Bell et al., 1980). The El Bordo las Tinajas *A. herbert-smithii* population in Nicaragua had the same chemicals in its seeds (C. Stirton and R. Nash, pers. comm.). These same compounds were also found in the seeds and leaves of five other species of *Ateleia*, in the leaves and seeds of *Cyanthostegia matthewsii* (Benth.) Schery, and in the leaves of *C. weberbaueri* (Harms) Schery (R. Nash, pers. comm.). This allogeneric similarity reinforces the suggestion (Rudd, 1968) that *Cyanthostegia* and *Ateleia* are closely related (see Discussion). The functions of these compounds are unknown, but they are at least a part of the defensive repertoire of the seeds and leaves, both of which are eaten only by monophagic insects (see below). These chemicals are not, however, the cause of rejection of *A. herbert-smithii* seeds by *Liomys salvini*, a native rodent and seed predator at Santa Rosa (see below). However, since this rodent eats many species of dry forest seeds that are known to contain chemicals toxic to other animals, there remains the possibility that the uncommon amino acids in *A. herbert-smithii* seeds are effective defenses against other vertebrates.

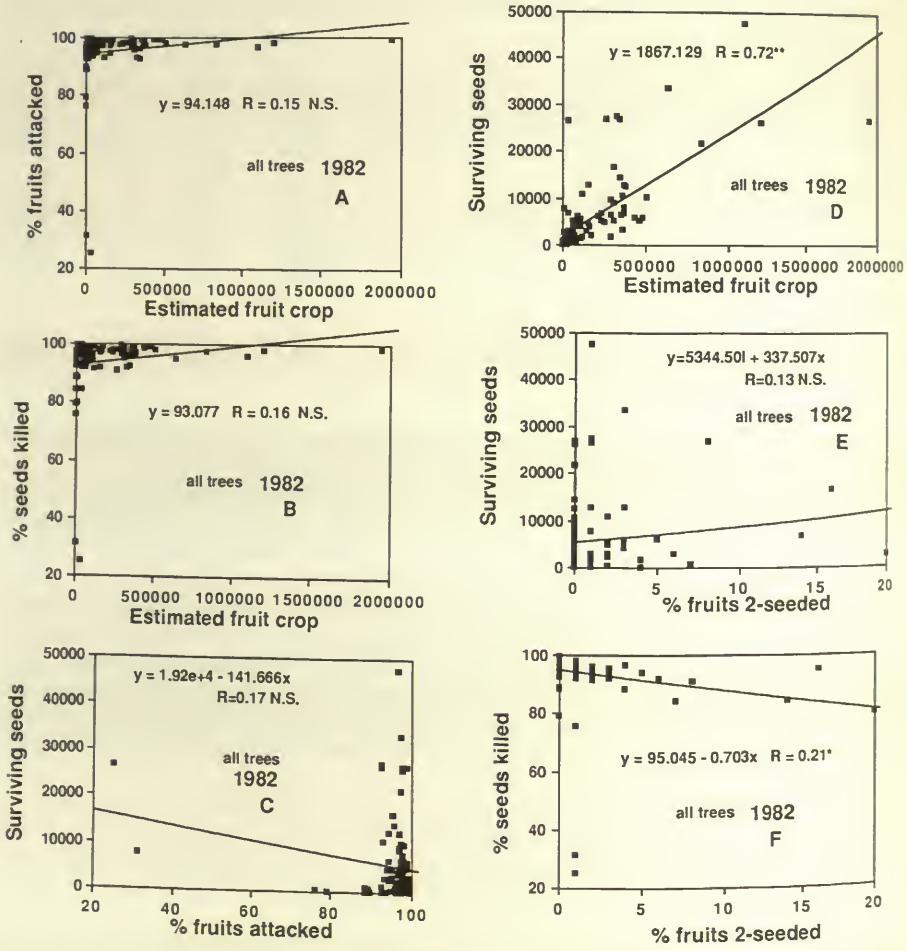


FIGURE 8. Relationships of fruit, seed, and seed predation (by *Apion johnschmitti*) parameters for all *Ateleia herbert-smithii* trees sampled in 1982. Same locale as Figure 1.

DISPERSAL

The indehiscent flat and dry mature fruits (autogyros, terminology of Augspurger, 1986a) of *Ateleia herbert-smithii* (Figs. 6, 7) are dispersed by the strong winds during the first half of the dry season at Santa Rosa (the Nicaraguan *A. herbert-smithii* population also experiences these early dry season winds; C. Hughes, pers. comm.). There is no suggestion that there is secondary dispersal (or postdispersal seed predation) by animals after wind generates the seed shadow. At Santa Rosa, no animals, including livestock, show interest in consuming the small and papery fruits while they are on the tree or after they have been dispersed. However, C. Hughes reported that cattle in Nicaragua avidly consume the fallen dry fruits; they must be very hungry cattle.

Fruits containing intact seeds are carried from zero to at least 100 m downwind from the crowns of 20-m-tall trees. When the parent tree is standing in open pasture, wind carries the fruits much farther than when the parent tree is in forest; the forest acts as a filter that literally rakes the fruits

out of the air and also creates turbulence that shortens the distance that a falling fruit moves. The fruit is released into the wind when the base of the fruit snaps off at about the point of contact with the dried flower calyx. This occurs on hot and dry days and when winds are strong (gusts at Santa Rosa may reach 80 km/hr.); at night the humidity rises, making the dry fruit tissue flexible and unbreakable by wind or breeze. If the day is cloudy, the fruits are commonly moist enough that even a strong wind does not detach them. If a particular dry season has an occasional sprinkle, much cloudy weather, and only sporadic winds (as occurred in 1983), there will still be large numbers of dried fruits hanging on the trees in the last half of the dry season. These fruits turn soggy and rot with the first rains, and finally fall from the tree as they decompose.

Seed shadows at Santa Rosa are impossible to map by merely recording the location of the indehiscent dispersed fruits. This is because nearly all fruits contain dead (beetle-attacked) seeds; the few fruits with viable seeds are heavier and therefore not evenly distributed among the dead fruits. The *Ateleia herbert-smithii* fruit shadow that was described by Gladstone (1979) was irrelevant to the seed shadow, since greater than 90% of the fruits whose locations he mapped had had their seeds eaten by weevil larvae several months before the fruits fell from the tree. I assume that as the variation in seed weight increases in the crop, a wind-generated seed shadow will be more homogeneously distributed over the surface (Janzen, 1978), but this has not been empirically examined for *A. herbert-smithii* (but see Augspurger, 1986b, for an example with the rainforest legume *Platypodium*).

SIZE OF FRUIT CROP

The fruit crops produced by an individual *Ateleia herbert-smithii* varied widely in size among years (Fig. 9, Appendix I). This variation had a number of causes: 1) No female flowers (or male flowers) were produced by the population in odd-numbered years from 1973 through 1983, and therefore seed crop sizes were zero in successive even-numbered years (1974 to 1984, inclusive). 2) Even in the years of population-wide flowering (even-numbered years from 1974 through 1984, and 1985), some adult individuals bore no flowers and therefore bore no fruits. For example, tree 177 bore fruit crops of approximately the following sizes in population-wide flowering years: 153,761 (1976); 0 (1978); 188,416 (1980); 468,545 (1982); 0 (1984); 5,756 (1985) (Appendix I) (it bore no flowers in any other years from 1975 to 1985). In 1974 and 1978 it did not flower, but in each of the other even-numbered years it had approximately equal-sized, very large flower crops. 3) A given tree produced different-sized flower crops, and therefore different-sized fruit crops, among years. Tree 11 flowered in all population-wide flowering years, but in 1984 the flower crop was at best 10% of the usual size and all the flowers were shed even in the few cases where they set fruit. 4) Equally large flower crops in successive flowering years on the same tree (as well as on different trees) produced fruit crops of very unequal sizes, For example, tree UM produced the following successive fruit crops from approximately equal-sized flower crops: 646,700 (1978); 426,136 (1980); 1,204,000 (1982); 5,642 (1984); and 565,200 (1985) (Fig. 9). The severe reduction in fruit production in 1984 was due to about two weeks of cool rainy weather during UM's flowering period. However, the above kinds of variation did not obscure the obvious pattern that really large fruit crops were always on fully insolated crowns (and that small crops could occur on a crown in the full sun as well as a shaded crown).

In addition to variation among trees, there also was patterned variation within a crown. Although neither stressed nor documented here, it was obvious that certain major and minor branching systems within a tree crown characteristically bore large or small numbers of fruits per infructescence. For example, the infructescences that had more than 500 fruits on tree 11 in 1982 (Fig. 14; Appendices VI, VII) were usually on a large horizontal branch projecting out of the northern part of the crown. Sometimes this variation was related to the physical environment; north sides of crowns bore fewer fruits (and flowers) than did southern sides, branches on the outer surface of the

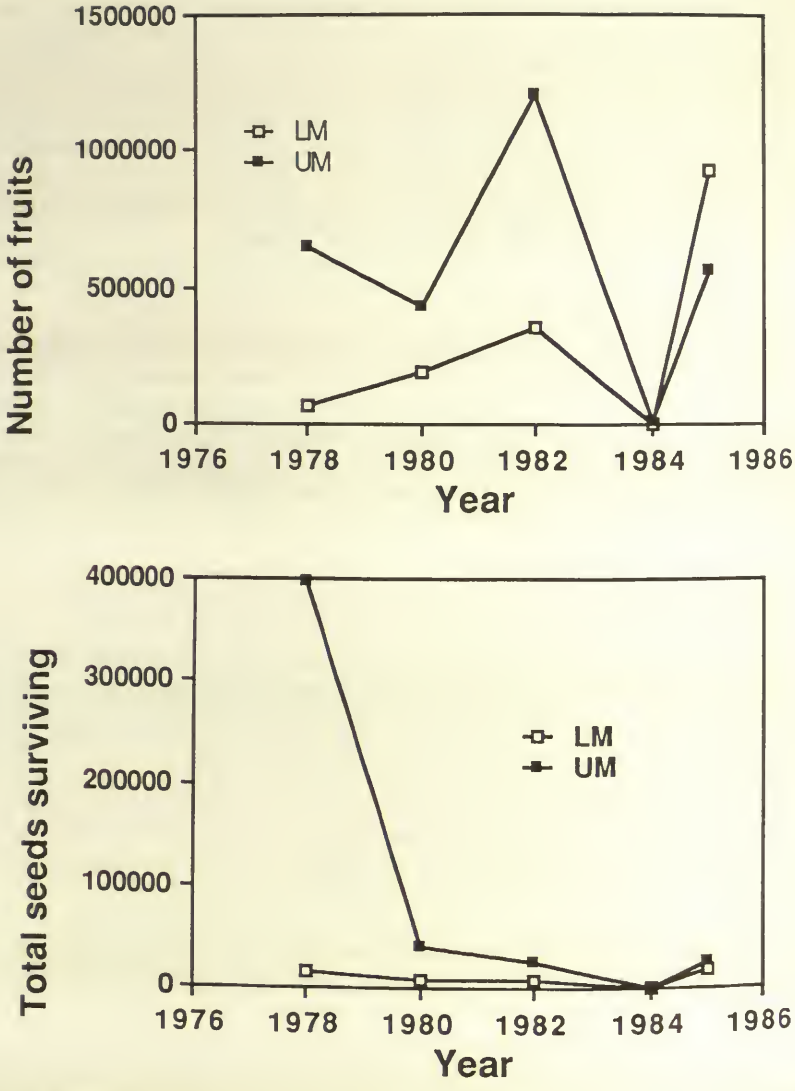


FIGURE 9. Relationship of fruit crop sizes (upper graph) to total numbers of surviving seeds (lower graph) for *Ateleia herbert-smithii* tree LM and UM throughout the census period. Same locale as Figure 1.

crown bore more fruits than did those within the crown, and branches shaded by other tree crowns bore few or no fruits. However, it also occurred that major adjacent branches in seemingly identical physical circumstances characteristically bore very different numbers of fruits.

Female crowns usually bore ten to many more flowers for each fruit that developed. There were some clear cases of fruit set limitation by lack of pollination. For example, when female flowers opened during cool and rainy days (cool and rainy spells are usually intermingled with sunny and breezy days in late October and early November), they were usually shed a few days later. There was no suggestion that these shed flowers were pollinated. The cause is obvious in the field. Not only are cool and rainy days at Santa Rosa relatively free of wind, but the rain probably sweeps the

air and foliage clean of the pollen reservoir that had accumulated from previous sunny and breezy days. Furthermore, the male trees do not mass-flower except on warm, sunny, and dry days (see below for details). Such days are breezy to almost windy and herald the beginning of the dry season. In years when the dry season begins early, much of the *Ateleia herbert-smithii* flowering period is warm, dry, and breezy. In other years, such as 1984, weeks of cloudy and lightly rainy weather occur during the *A. herbert-smithii* flowering season and many open flowers were shed by females, apparently because of lack of pollination. At Santa Rosa, a rainy spell in late October or early November usually lasts 3–10 days. Once it has passed, the weather turns clear, warm, and breezy. Males begin to flower again, and many of the female flowers are once again retained as developing fruit.

While *A. herbert-smithii* do abort open female flowers and some young fruits (of various ages) before there is a conspicuous increase in seed size, nothing about the timing of fruit abortion suggests that it is a response to oviposition in the young fruit by the seed predator weevil (see below).

Ordinary adult *A. herbert-smithii* bore 5–100 thousand fruits during ordinary fruiting years (Fig. 8a; Table 1; Appendix I). However, a tree that usually bore crops of this size also bore as many as 0.3–2 million fruits in a year in which it has an exceptionally high fruit set (some of the trees with large crops in Fig. 8a had fruit crops of quite ordinary size in other years). Furthermore, there were certain trees that regularly had fruit crops of this very great size. It is presently impossible to evaluate the actual cost of such a large fruit crop, since usually more than 80–90% of the seeds were destroyed by weevils (Table 4) as they began to fill. Furthermore, there is no way to know at present what fraction of the resources of a mature seed are moved into the growing seed's tissue as it is being eaten by the weevil larva (see below). I suspect, however, that the cost to the mother of a crop in which 90% of the seeds are killed by the weevil is well less than 20% of the cost of maturing that entire crop of seeds, because the weevil is so small that it uses only a small fraction of the material needed to fill a seed. The trees that have escaped intense seed predation by the weevil in a given year and set large crops (e.g., various trees in 1974, 1976, and 1978; tree 296 in 1982; Appendix I) demonstrated that a tree can have sufficient reserves to mature a very large crop of seeds. This is also suggested by the fact that, owing to the absence of the weevil, *A. herbert-smithii* in Nicaragua matures very large seed crops (C. Hughes and B. Styles, pers. comm.).

It is tempting to suggest that the 1985 crop was generally small (Table 1) because it immediately followed the 1984 crop (without an intervening year of accumulation of reserves). Likewise, it is tempting to suggest that the small 1984 crop (in great part due to rainy weather in the 1984 flowering season) resulted in the trees having enough reserves to fruit again in 1985 but not enough to make really large crops.

FRUIT CROP SYNCHRONY

All adult female *Ateleia herbert-smithii* in Santa Rosa produced no flowers in 1973, 1975, 1977, 1979, 1981, and 1983, and flowered (and fruited) in 1974, 1976, 1978, 1980, 1982, and 1984 (Table 1, Appendix I); however, in 1985 they also flowered, thereby destroying what had appeared to be the trait of perfect population-wide fruiting synchrony. (In the 1986 flowering season, about 1% of the male trees had a very few small inflorescences on them in Santa Rosa, but no female trees bore flowers, and there were no seeds.) This flowering also destroyed the temptation to postulate a pheromonal control or magical internal calendar for the entry of newly reproductive teenage trees into perfectly biennial flowering synchrony of a dioecious tree population. The male *A. herbert-smithii* adhered perfectly to the same flowering schedule as that of the female *A. herbert-smithii*, except that a single large adult male in full heavy flower was found in 1979, and a single different male had a weak flower crop in 1983. In addition to the males growing next to the individually numbered female trees that were under intense scrutiny during the 1974–1984 flowering seasons,

at least 200 other male adult *A. herbert-smithii* live in the study sites that I frequent and are sufficiently conspicuous that I would have noticed them had they flowered in the odd-numbered years other than 1985. In other words, approximately 300 adult male trees under close observation made only two flowering "errors" in 10 years.

The environmental cueing system used by *Ateleia herbert-smithii* is not obvious, and it must occur during the several rainy season months before the buds appear, unless the plant has something that functions as a memory. The 2–8 mm undeveloped inflorescences are obvious on the trees as early as the August before the October–November flowering; however, in August these buds cannot be more than a few months old. The inflorescences originate in the leaf axils of the leafy branches produced at the beginning of the rainy season (April–May) of the flowering year.

In the Nicaraguan population, the *A. herbert-smithii* flowered in 1981 (mature fruit collected in the 1982 dry season) and did not flower in 1982 (C. Hughes, pers. comm.). In 1984, this population flowered again (it bore mature fruit in 1985; D. Stevens, pers. comm.). It also bore mature fruit in 1986. This means that it flowered in both 1984 and 1985, just as did the Santa Rosa population.

VEGETATIVE SEXUAL DIMORPHISM

Most adult male and female *Ateleia herbert-smithii* can be distinguished by their crown profile; males tend toward multiple trunks and many long, thin, and semivertical branches, while females tend toward single trunks with stouter and more horizontal branches (Figs. 1, 2). The developmental cause is at least partly because female infructescence-bearing branch ends (Fig. 6) die back farther and the next year's new female branches do not elongate as much as do next year's male branches. Adult female *A. herbert-smithii* are usually 2–5 m shorter than are adjacent male trees of the same dbh and approximate age. On a fruiting year, male trees retain a dense crop of green leaves longer into the end of the rainy season than do the females, which begin to yellow and drop their leaves about the time that the fruits are beginning to contain full-sized seeds (late November or December, which is the last month of the rainy season or the first month of the dry season). There is also more biomass of wood in the crown of a male than in the crown of a female tree of the same dbh.

FLOWERING

Female flowers

Female *Ateleia herbert-smithii* flowers are borne on linear branchlets in terminal multibranched inflorescences (Figs. 3, 5); there are fewer flowers and flowering branchlets per female than per male inflorescence. The (green) branch axis immediately proximal to the inflorescence bears a shaggy and subtending rosette of that year's leaves (Fig. 3). The next year's branch will appear from one of the axils of these leaves. While I am not certain whether the *A. herbert-smithii* inflorescence should be termed literally "terminal," for all practical purposes it is terminal and dies back to the area of the axils of the subtending rosette of leaves.

Flowering branch ends occur over the entire surface of the crown, but branches that have their leaves fully exposed to the sun often make larger inflorescences (just as crowns that are fully insolated make the largest fruit crops). Maximal branch elongation (and therefore maximal leafing) appears to require full insolation; shaded portions of the crown die away or bear very sparse foliage and may entirely lack flowers. The flowering branches inside the crown are in those areas of the crown where it is not very thorough in casting shade. On the other hand, *Ateleia herbert-smithii* crowns are conspicuously diffuse (Figs. 1, 2), and enough light penetrates to allow some leafy and flowering development well within the crown.

The flowers open progressively from the base to the tip of the inflorescence. A few new ones

open each day. The entire inflorescence uses 1–2 weeks to open all its flowers (and seems to slow down in cool weather). The female flower has a comparatively huge and discoid stigmatic surface (1–2 mm wide) that is directed upward and fully exposed to the air (Fig. 5). The style is shorter than the stigma is broad (this statement is, however, somewhat subjective because the style merges with the ovary). The twisted and dwarfed anthers on their short filaments do not project above the stigmatic surface (Fig. 5). The anthers do not generally open; they also contain what appears to be collapsed pollen and often fall off within a few hours of when the flower opens.

The calyces are pale green with slightly reddish hairs, and the single petal is white. The petal is reflexed backward (but not as severely as in the male flowers, Fig. 4) so that it approximates a shallow hood behind the stigma (Fig. 5). I suspect that its scooped shape and position creates local air turbulence, which results in more pollen being deposited than would be the case with more laminar air flow over the flower or inflorescence surface. The inner surface of the petal is impressed with the shape of the stigma and other floral parts that were pressed tightly against it in the bud (as can be seen in the male flowers in Fig. 4).

During the 1976 and 1978 flower crops, I enclosed ten inflorescences in window screen boxes, after having first removed all the open (distal) flowers and (proximal) developing fruits from the inflorescences. In a parallel set of equal-sized experiments, only the newly opened flowers were removed. The flower buds on all the screened inflorescences continued to open and set seed just as did those on adjacent altered and unaltered unscreened control branches. A second set of inflorescences was treated in the same way, but the experimental inflorescences were enclosed in light paper bags instead of screen. The flowers in the bags opened normally but did not set fruit and were shed a few days after opening. When these experiments are coupled with the observations below, the conclusion that *Ateleia herbert-smithii* is wind-pollinated is inescapable.

Bees visit only the male flowers (see below). During many hours of observation of female *Ateleia herbert-smithii* inflorescences, I have never seen an insect visit a female flower (except for the weevils that occasionally walk over the flowers in search of young green fruits and the occasional hunting wasp that lands on a flower while searching for caterpillars on the foliage). No nectar or other potential reward is produced by the female flowers. The female flowers are odorless to me.

Linear female flowering branchlets bear as few as 20–40 flowers and as many as 200–1,000; a terminal inflorescence usually bears 1–50 times this amount. A representative terminal inflorescence usually bears about 1,000–4,000 flowers but can bear as many as 20,000. Two thousand flowers commonly produce 25 to 300 fruits, but there may be as few as one and as many as 2,000 fruits on a branch end. The detailed observations have never been made, but a ratio of about 20 flowers to each fruit that is set in an ordinary year appears to be close to the average for an ordinary year. A medium-sized adult tree with 50,000 fruits on it would have had about 1,000,000 flowers in an ordinary year. In normal years, the very high variation in numbers of fruits per branchlet and per terminal inflorescence appears to be due in great part to variation in the amount of pollination and fertilization below the limit imposed by the size of the flower crop.

As described above, it is clear that weather conditions can influence the degree of pollination of female flowers. The amount of resources also limits fruit set. There is a suggestion that if a flower-bearing branchlet has set several hundred fruits at the base of the inflorescence (because of good weather when the first set of flower buds opened), it may shed the terminal buds without opening them; alternatively, I have observed that if the basal flowers do not set fruit, the female flower buds continue to open all the way to the end of the branchlet (and the terminal flowers can set fruit; Fig. 5).

A tree's fruit crop may be sired by any number from one to hundreds of male trees. Two large female *Ateleia herbert-smithii* at Santa Rosa grow in a pasture 400 m from the nearest adult males, which are downwind, and 600–700 m from the nearest upwind males. Nevertheless there must be sufficient pollen in the air, since they produce crops of over 100,000 fruits. In relatively intact secondary successional forest at Santa Rosa, a circle of 500 m radius around a female tree may contain as many as 50 male trees, most of which will produce 1–3 pulses of flower openings

sometime during the female's flowering period. However, the leafy forest is also a giant pollen filter, and pollen flow may not be able to move as far as the distances suggested above. Despite this caveat, the usual density of the population is such that a single fruit crop is very likely to be sired by substantially more than one male.

Male flowers

Male inflorescences are longer, more branched, and more upright (Figs. 3, 4) than are female inflorescences (the latter give the impression of being stubby and more splayed out, presumably for the same reason that the stigma is enlarged). More of the leafy branchlets in a male tree bear inflorescences at their ends than is the case in female trees, but the differences are not great.

Male flower buds are very similar to those of female flowers. The greenish calyx forms an open-topped globe, with the top plugged by the curved blade of the single petal. When the flower opens, the calyx spreads out into more of a cup, the petal pops out and arches back to where the concave side points upward, and the stamens extend separately well above the calyx margin (Fig. 4). Open male flowers look superficially like female flowers, but the rudimentary ovary and style is hardly more than a shrunken nub hidden between the bases of the thick and clustered anther filaments. There are 8–14 white anther filaments per flower (10 being a very common number) and each appears to bear a fully developed and normal-appearing yellow anther. About half of the filaments are about 1–2 mm shorter than the others. The longest anthers are about 4–5 mm long, giving the impression of two tiers of anthers.

The anthers open along one side and evert the pollen, which is loose and dry. The single petal arches back from the open flower as a tiny reflexed hood converted to a scoop, and the mouth of the scoop usually opens upward and tends to fill the spaces between the flowers. I suspect that it serves as a pollen basket (pollen accumulates in the standard) until a gust of wind occurs that has sufficient strength to launch the pollen (rather than let it sift gradually to the ground as the flowers open and anthers dehisce). The curved surface of the petal may also generate air turbulence around the inflorescence, as suggested for the female flowers. (The hood on the male *Ateleia gummifera* flower drawn in fig. 5 in Dillon, 1980, is probably only partly extended and flexed) *Ateleia herbert-smithii* pollen is tricolporate, with distinctive reticulate ornamentation (Fig. 21), very similar to other *Ateleia* pollen, and resembles typical generalized papilionaceous legume pollen (K. Ferguson, pers. comm., based on male flowers from Santa Rosa *A. herbert-smithii* raised in the greenhouses at Kew Gardens by C. Stirton, 1982–1986).

When a mass of male flowers is open, the air is filled with a strong fragrant odor similar to the odor of sphingid flowers (e.g., *Alibertia, Randia, Exostemma*) but more musky. While this odor suggests that *Ateleia herbert-smithii* was once pollinated by moths, it might be a simple by-product of some other process. No nectar was visible or detectable by taste in any flowers (male or female), and the multitude of bees (Fig. 4) visiting the male flowers did not push their mouthparts down into the central interior of the flowers; instead, they very rapidly collected pollen.

Male flowers open according to a different pattern than do female flowers. For many days many buds on a given tree remain full-sized and apparently ready to open, and then on a warm, dry, and breezy morning, they open synchronously shortly before dawn. As many as half of the flower buds may open on this one morning. If this warm day follows a series of cloudy and cool days, numerous males may flower on the same morning. For example, 24–30 October 1985 was rainy, darkly cloudy, and calm. October 31 cleared up and by sunset was breezy and warm. On 1 November at least 11 large adult male trees out of 18 in one study area had huge crops of open flowers, all being heavily visited by pollen-collecting bees. However, the rain started again about 3 hours after dawn and washed masses of flowers and pollen onto the ground below the trees. November 2–4 remained cloudy and cold with afternoon rain (such a weather event is locally termed a "temporal"); there was no sign of flowers and no bee activity at the trees. November 5 dawned clear, and again

numerous males had moderate crops of open flowers that were being visited by bees. Throughout this period, new flowers opened daily on the female trees, although I was left with the impression that not as many opened during the gray and cool weather as on warm days. The weather-generated synchrony among the males is not conspicuous if the weather is continuously warm and breezy; however, a detailed study of hundreds of plants over a large area might well disclose subtle synchrony. In warm breezy weather, a given tree retains its within-crown synchrony and behavior of flowering at intervals. I suspect that the within-crown synchrony is functional in satiation of pollen-collecting bees.

Copious amounts of pollen are released into the air when a pulse of opening male flowers occurs. If a flowering branch is lightly shaken, the air is filled with conspicuous clouds of golden pollen that float away in the air column, just as is the case when a pine tree branch laden with opening male cones is shaken. It is obvious that not only are cloudy and cool (and therefore wind-free in this part of Costa Rica) conditions bad for pollen flow, but morning rain washes a tree's pollen pulse to the ground from the upward directed and open flowers, converts the inflorescences into a soggy quagmire, and converts the entire forest into a monstrous tanglefoot trap for pollen by wetting the vegetation.

Ateleia herbert-smithii male flowers are very heavily visited by female social bees and largely ignored by solitary bees except for the occasional *Xylocopa* and halictid bee (no male bees visited the flowers, since they did not produce nectar and social bees do not mate at flowers). However, it is also possible that at this time of year, when only *Lonchocarpus minimiflorus* and very few other bee-pollinated woody plants are in flower at Santa Rosa, there are relatively few adult solitary bees active in the habitat. The social bees collect enormous amounts of pollen. From dawn to mid morning, an *A. herbert-smithii* male tree with a newly opened flower pulse has a greater biomass of bees in it than I have observed in the flowering crown of any other species of tree in Santa Rosa. The bee biomass is species-rich. For example, five minutes of sweeping an insect net through the air immediately over the newly opened male flowers on lower branches (3–4 m from the ground) of tree 141 on 30 October 1982 yielded the following social bee species (all were females that were collecting pollen from the flowers): *Melipona beechei* and *Trigona pectoralis, silvestriana, jatiformis,* aff. *cupira, testaceicornis perilampoides, fulviventris, angustula, capitata zexmeniae, fuscipennis, dorsalis, mellicolor, buchwaldi, frontalis,* and *buyssoni* (determinations by D. Roubik). This is the entire known native social bee fauna of Santa Rosa National Park, plus one new addition. This bee sample was taken prior to the arrival of the feral African honey bee *(Apis melifera)* in the 1984 dry season, and I have since observed this species to collect *A. herbert-smithii* pollen as well.

The male flowers are shed in the afternoon, night, and day following a flowering pulse. The ground beneath the tree may be as much as 5 mm deep in fallen flowers. As many as half of the flowers on a tree may have opened in such a pulse. However, the synchrony is not perfect within a crown, and a dribble of newly opening flowers continues for many days after. A week or more later there may be a second, but less intense flowering pulse. Following this, flowering continues to dribble on for weeks. It usually takes about 3–5 weeks for a male tree to exhaust its flower crop, but it goes faster if the weather stays continually warm, sunny, and breezy.

The bulk of the male trees flower in the late October–November period during which the bulk of the female trees open their flowers. However, there is a long tail on the distribution. A few individuals are still producing both male and female flowers in small numbers as late as mid January, and there are a few males that have their flowering pulses as late as mid December. Once the flowers have dropped, a late-flowering male is not easily recognized; however, late-flowering individual females are easily recognizable at a later date by the comparatively less mature state of their fruits (see also below with reference to escape from seed predation).

A large emergent male *Ateleia herbert-smithii* produces an enormous number of flowers. For example, on the morning of 30 October 1982, *A. herbert-smithii* 141 opened an estimated 11,800,000 flowers (37.52 kg of wet mass of flowers after most of the pollen had been shed) on 4,000 terminal inflorescences. This was its second mass flowering episode; on 21 October 1982 the

first flowering episode of tree 141 produced an estimated 4,712,000 flowers. After both of these flowerings, there were still an estimated 2,059,000 unopened buds on the tree; many of these flowers opened over a 2–3 day period starting on 6 November 1982. This estimated total of 18,570,400 flowers represented about 59 kg of wet mass (14.9 kg dry mass) after the pollen had been shed. Exceptionally large and fully emergent male trees may bear as many as ten times the number of flowers recorded in the example above; 3-m-tall juveniles may bear as few as 1–5 flowering terminal inflorescences, each with 1,000–3,000 flowers.

Juvenile trees

Young female trees sometimes have a few male flowers scattered through their inflorescences. The reverse case has not been encountered.

In nature, the age at first flowering (males and females) of fully insolated saplings is about 5–6 years. They are 3–4 m tall at this time. However, in the Oxford University (England) heated greenhouses, B. Styles and C. Hughes planted Nicaraguan seed on 2 February 1985, and the first plant opened flowers on male inflorescences borne on a single branch on 5 December 1985. Likewise, 2-m-tall 3-year-old *Ateleia herbert-smithii* seedlings/saplings grown from Santa Rosa seed at Kew Gardens produced male flowers. Since at Kew the plant was growing almost year-round, it had about as many months of growth before its first flowers as does a wild Santa Rosa plant that first flowers at an age of 6–7 years but experiences only a 6-month growing season each year. The Oxford plants, however, displayed inexplicably early flowering.

Young males often produce smaller inflorescences with fewer lateral branchlets than do adult males (as was also observed by Styles, Hughes, and Stirton in the English greenhouse trees). Young females, however, tend to make large inflorescences and even set large numbers of seeds per inflorescence, but they produce relatively few inflorescences.

Heavily shaded *Ateleia herbert-smithii* Santa Rosa saplings produce only a few leaves each year and barely increase in size. They do not flower even after 6–7 years of growth, and I suspect they eventually die unless they become insolated as the result of a tree fall.

IV. Seed Predation

Pre-dispersal seed predation by weevils

Natural history of weevils

As a population and within most individuals' crops in most years, *Ateleia herbert-smithii* sustains the greatest percent pre-dispersal seed predation (Figs. 8, 9, 13–19; Tables 2–4, 6; Appendices I, V–VII) that I have witnessed for any tropical tree. It is so great that the positive correlation between the fruit crop size and the absolute number of surviving seeds (Fig. 8d) is severely obscured (e.g., Fig. 9) because the fruit crop sizes are enormous in comparison with the absolute number of surviving seeds in a crop. The bigger the seed crop borne by an *A. herbert-smithii* crown, the larger will be the absolute number of surviving seeds that it generates (e.g., Fig. 8d), but the variation among trees and years is very great. A small fruit crop can contain a relatively large absolute number of viable seeds and an enormous fruit crop can contain almost no viable seeds (e.g., Fig. 8d). Virtually all of this seed predation is committed by a 2-mm-long black and gray curculionid weevil (Fig. 6), *Apion johnschmitti* Whitehead (Whitehead, 1985). The weevils may be easily observed by close scrutiny of very young *A. herbert-smithii* infructescences (early November) day or night. They also are found climbing through male inflorescences. There is, however, no aspect of their natural history that suggests that they may be regular pollinators of *A. herbert-smithii* by carrying pollen to the female stigmas (although this certainly could occur if they had appropriate

TABLE 6. Percent of the surviving *Ateleia herbert-smithii* seeds that survived because they were in two-seeded fruits, 1974–1985 in Santa Rosa National Park.

	1974	1976	1978	1980	1982	1984	1985
Average	13.41	10.18	not available	6.67	12.91	5.58	23.59
s.d.	22.6	19.57		9.37	19.39	8.34	21.16
n	20	33		25	101	37	60
Maximum	71.43	66		37.58	78.71	29.44	79.7
Minimum	0	0		0	0	0	0
Range	71.43	66		37.58	78.71	29.44	79.7
Median	0	0		3.2	5.13	1.35	17.67
Skewness	1.29	1.62		1.69	1.99	1.36	0.79
Kurtosis	0.17	1.11		2.44	3.1	0.48	−0.28
Probability distribution normal	0.047	0.007		0.117	0.006	0.043	0.149

behavior). I have never even seen one on a newly opened flower, even though they are common on the adjacent new green fruits. I suspect that the weevil uses the strong odors of *A. herbert-smithii* vegetation to locate the trees and does not discriminate between male and female plants. Additionally, it may be feeding on the pollen when it is on male inflorescences.

The weevil walks across the green fruits and oviposits on the lateral surfaces, in the vicinity of the seed. Oviposition occurs when the expanding seed is not much more than 1 mm wide (i.e., within 4–10 days after flowering). The fruit is 5–10 mm wide at this time. I have not determined if she breaks the surface of the fruit in the act of ovipositing. She walks away from the fruit after ovipositing on it. Inferring from the oviposition regime of other weevils (Curculionidae) and bruchids (Bruchidae), she lays 50–200 eggs in a few weeks and then dies.

The first instar larva mines through the fruit wall, lodges partly inside the seed at the point of intersection of the funiculus with the seed, and appears to be eating the embryo as it develops. This means that the larva may have little or no contact with the secondary and potentially defensive compounds found in the mature seed (see below under seed chemistry). Little or none of the seed coat is eaten, and it continues to expand to nearly full-size (Figs. 6, 7). That the larva can feed on the embryo as the embryo grows (or attempts to grow) suggests that the parent plant does not physiologically distinguish between an intact growing seed and one with a weevil larva in it. There is no suggestion that *A. herbert-smithii* fruits with weevil larvae in them are aborted or shed by the plant earlier than are those with intact seeds in them.

There is never more than one larva per fruit. Either the weevil avoids oviposition in those fruits in which oviposition has already occurred, or the first larva to arrive consumes the latecomers (the latter kind of cannibalism is committed frequently by seed predator bruchid beetle larvae, but it is only indirectly suggested in this case). The weevil larva pupates or is ready to do so within about 4 weeks after the egg was laid (in early November or late October). If the attacked green fruits are picked from the tree (in early December or later) and begin to dry up, within 2–10 days the weevil completes development and emerges through a small circular exit hole cut by the adult weevil through the side of the fruit (Fig. 6). At this time in the season, unattacked seeds are full-sized and solidly filled with green embryo and endosperm (Fig. 6). However, if the attacked fruits are left on the tree, the weevils emerge as late as early January, at about the time that the fruits on the tree begin to dry and turn from bright green to yellowish.

After emerging, the weevils wait as adults in the habitat for 10 or 22 months before the next *Ateleia herbert-smithii* crop appears. If there was a crop failure for three years in a row, perhaps the weevil would have to live 34 months. The larvae of this weevil develop in no other species of plant in Santa Rosa (this statement is based on the extensive and ongoing rearing of pre-dispersal seed predators from all potential hosts; Janzen, 1980). The waiting adults have never been located at Santa Rosa, but many other species of weevils and bruchids with similar biologies spend the waiting

period at Santa Rosa as quiescent adults in sheltered places (e.g., rolled leaves, crevices in tree bark, abandoned insect nests, etc.). These adults sometimes visit flowers, extrafloral nectaries, and new green foliage for moisture and food, but it is not known if the *A. herbert-smithii* weevil does this. No weevil larvae have been found in *A. herbert-smithii* flower buds (although flower buds of other legume species are often attacked by other species of weevil larvae, and an *Apion* has been found in *Ateleia arsenii* flower buds in Mexico [Whitehead, 1985]).

Sampling pre-dispersal seed predation

The intensity of pre-dispersal seed predation by the weevil is easy to determine and not labor-intensive, even for large samples. A fruiting branch end is cut from the tree and the 1–2-month-old fruits are stripped off. I then spread the translucent fruits on a glass or plastic sheet, with a strong light shining through from below. Intact seeds appear as solid black-green discs, whereas a seed containing a weevil larva (or its parasitoids) is semitranslucent gray and lighter green (Fig. 7). An experienced person with a high tolerance for boredom and a good stereo can thus survey thousands of fruits per hour for weevil damage (two-seeded fruits are also easily detected during this "candling"). Fruits that are still attached to the tree can also be examined (more laboriously) in the field by looking through the fruit held up against a beam of sunlight. Once the fruits are dried and the weevils have left, a rough minimum damage estimate can be obtained by counting fruits with exit holes (the "exit hole of a moth larva" in the *A. herbert-smithii* fruit wall in fig. 1 in Janzen, 1978, is in fact the exit hole of the adult weevil). Such a method, however, yields a serious underestimate if many weevils have died in the fruits or if there is a high rate of parasitism (hymenopterous parasitoids of the weevil larvae sometimes leave only tiny exit holes and sometimes die in the fruit). Mature dry fruits can also be scored for damage as described above with the immature fruits, but full-sized aborted seeds are difficult to distinguish from intact seeds when the fruit is examined by transmitted light.

Sampling fruits for weevil attack and determining fruit crop sizes has some subjective components but is moderately reliable. Fruit crop sizes were determined by directly counting the number of infructescences over 5–10% of the crown and then estimating how many such large areas there were in the crown. A sample of 20–60 infructescences was then cut haphazardly out of the tree crown by a climber or with a 6 m pole-pruner. The numbers of fruits in the infructescences were used to calculate a mean number of fruits per infructescence for each tree. Since there is only one infructescence per leafy branch end, the shorter word "branch" is used throughout this paper as synonymous with "infructescence." The numbers of fruits in each infructescence in this sample were counted directly at the same time that the fruits were candled for weevil damage. In the 1982 and 1984 crops, the fruits on three large trees were collected entirely and all fruits counted; the estimates were 92, 94, and 106 percent of the actual number of fruits. Very large samples were recorded for three other trees (Appendices V–VII). The infructescences were randomized and sampled in cumulative samples of 20, and from these the average number of fruits on a branch (Fig. 10), average percent of fruits attacked on a branch (Fig. 11), and average number of unattacked fruits on a branch (Fig. 12) were calculated. In most cases a sample of 40 infructescences is a more accurate representation of the true value than is a sample of 20. However, 20 gives a value within 1–2% of the mean in almost all cases, which is certainly accurate enough for the level of resolution of crop parameters discussed here.

In many of the comparisons below, I use the data from three very large samples (tree 181, 1982; tree 11, 1982 and 1985) for examination of within-crown processes. This eliminates worry about small sample sizes in interpreting processes and data. However, I should also add that the observations on the hundreds of other and smaller samples were unambiguously consistent with the results from the three large samples.

Throughout the description of weevil attack, I distinguish between seed death and fruit attack.

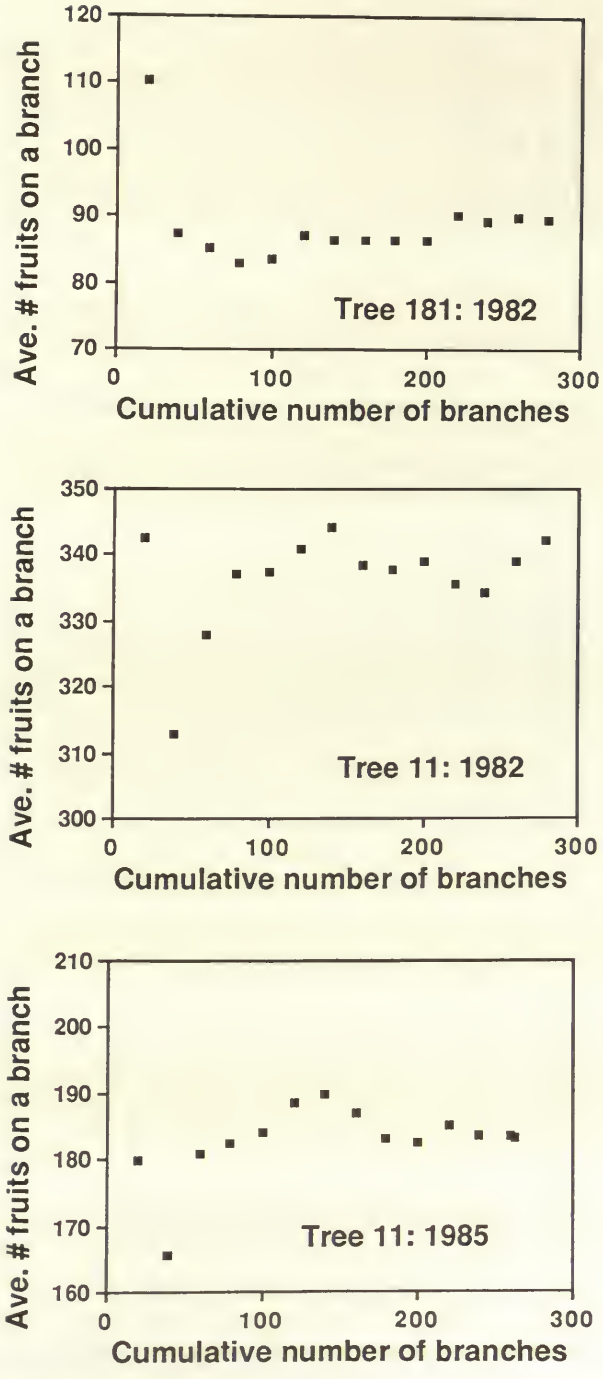


FIGURE 10. Relationship of average number of fruits on a branch (in an infructescence) to the cumulative number of branches sampled within three large samples of *Ateleia herbert-smithii*. Same locale as Figure 1.

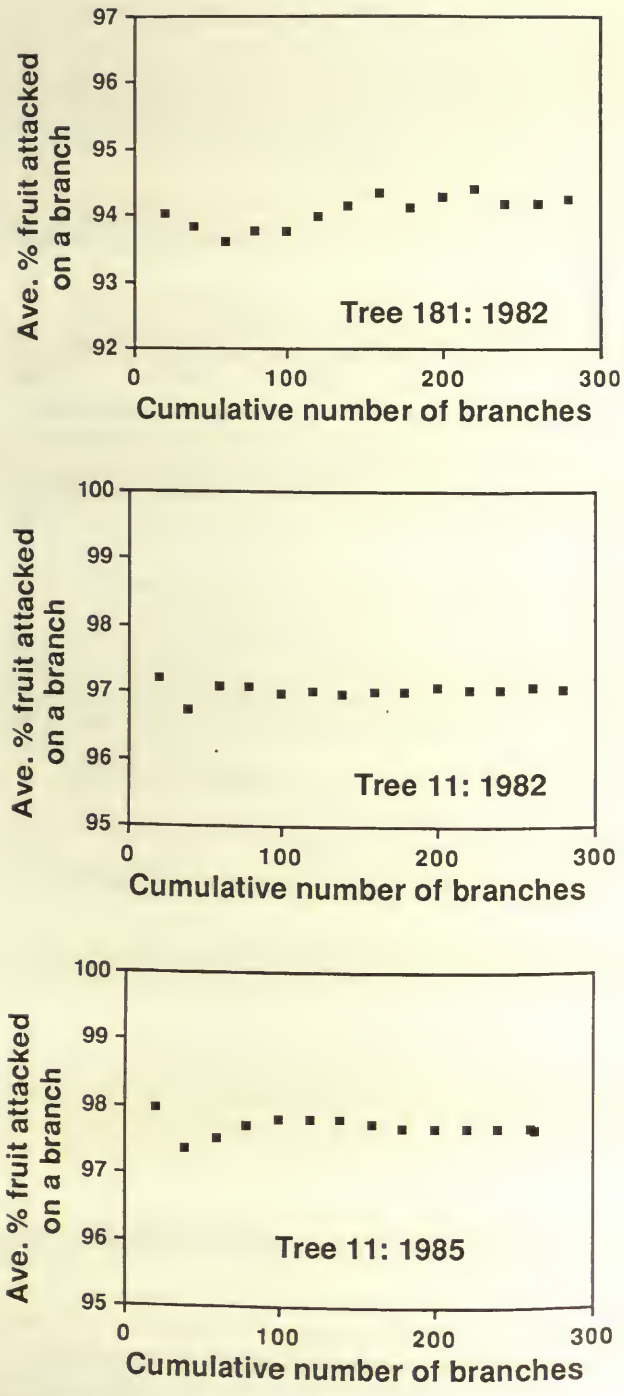
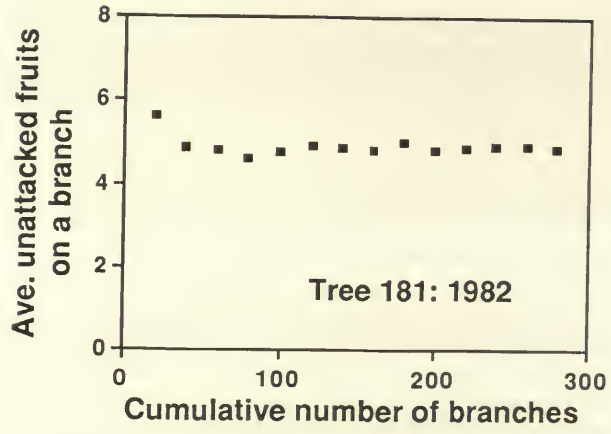
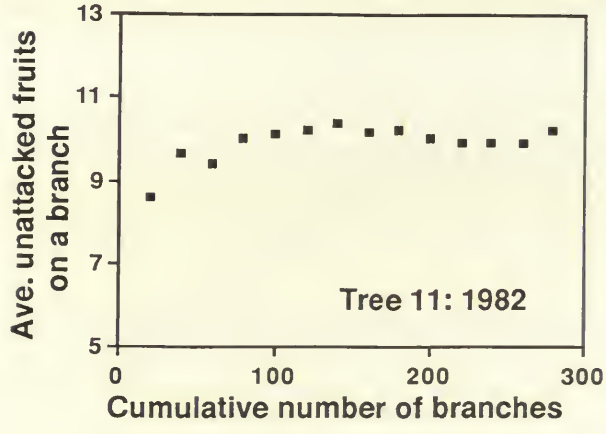


FIGURE 11. Relationship of the average of the percent of fruits attacked on a branch (in an infructescence) to the cumulative number of branches sampled. Same branches and locale as in Figure 10.





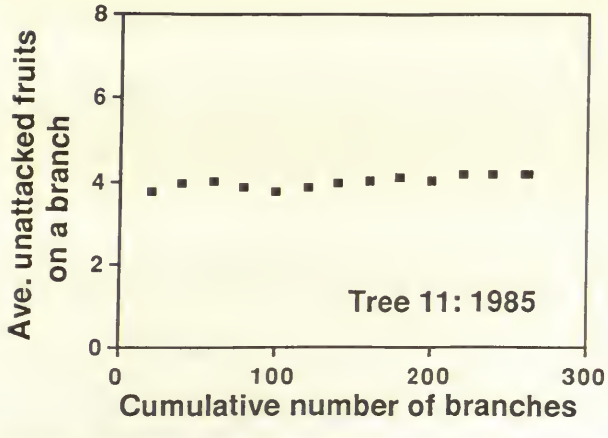


FIGURE 12. Relationship of the average number of unattacked fruits on a branch (in an infructescence) to the cumulative number of branches sampled. Same branches and locale as in Figure 10.

The former is determined unambiguously by observation. Only a minimum estimate can be determined for the latter, since a fruit may be attacked without its seed being killed. Also, observable fruit attack does not equal seed death because the fruit may be attacked but only one of a pair of seeds killed.

Intensity of pre-dispersal seed predation

The average percent seed predation by the weevil usually ranges from 80–98% over a large sample of trees (Table 4), but it is commonplace for individual trees to have more than 98% of the seed crop killed by weevils (Figs. 8b, 18; Appendix I). On rare occasions, a crown suffers only a moderate percent seed predation by the weevil (see next section below). The average of the percent of seeds killed by the weevil seems to increase from 1974 to 1985 (Table 4). The two highest percentages were recorded in 1984 and 1985 and could well represent the highest attainable given that the weevil makes some errors and is so abundant that there is no predator satiation (see below). I stated in Janzen (1978) that the *Ateleia herbert-smithii* at Santa Rosa did not flower in 1972 and 1973, and thus the 1974 low in percent predation could be the result of two successive years without a seed crop (meaning that the weevils that oviposited in 1974 had to have survived for 34 months). However, I have since come to have reduced faith in the source of the information that there was no flowering in 1972, so it is best to simply recognize a low percent mortality in the 1974 crop and leave the cause undetermined. At the high end of the scale, the high percent of seed mortality in 1984 and 1985 could be due to both small overall seed crops (= poor seed predator satiation) and high weevil survival during the single year between seed crops.

Causes of seed survival

The fruits that contained intact seeds were not clustered together at a point within an infructescence (Fig. 6), but rather were scattered among the fruits that had weevils in their seeds. The processes that led to surviving seeds were obviously multiple, and many of them co-occurred in the same seed crop; they ranged from weevil errors to population-wide seed predator satiation:

1. In 1-seeded fruits

a) A weevil can oviposit (and therefore probably render a fruit unsuitable for oviposition from the viewpoint of other weevils) but then her egg or newly hatched larva suffer developmental failure. If the failure of a weevil to develop in a marked fruit is the sole cause of a seed's survival, the percent of seed survival in trees with greater than 95% seed attack by the weevil is probably equal to the percent of weevil immatures that have developmental failure. In other words, seed predator satiation is not occurring at the high levels of percent of seed attack. Such failure should be randomly distributed over the branches unless a female largely confines her oviposition activities to a single infructescence and the eggs of certain females are more prone to developmental failure than are those of others. In the three large crops (Appendices V–VII), the number of fruits attacked on a branch was perfectly correlated with the number of fruits on a branch (Fig. 13). There was a conspicuous uniform increase in the number of unattacked fruits as the number of fruits on a branch increased (Fig. 15). Likewise, there was no correlation between the percent of fruits attacked on a branch and the number of fruits on a branch (Fig. 14). These results are consistent with a random distribution of weevil developmental failures among the branches, but could be caused by other processes as well.

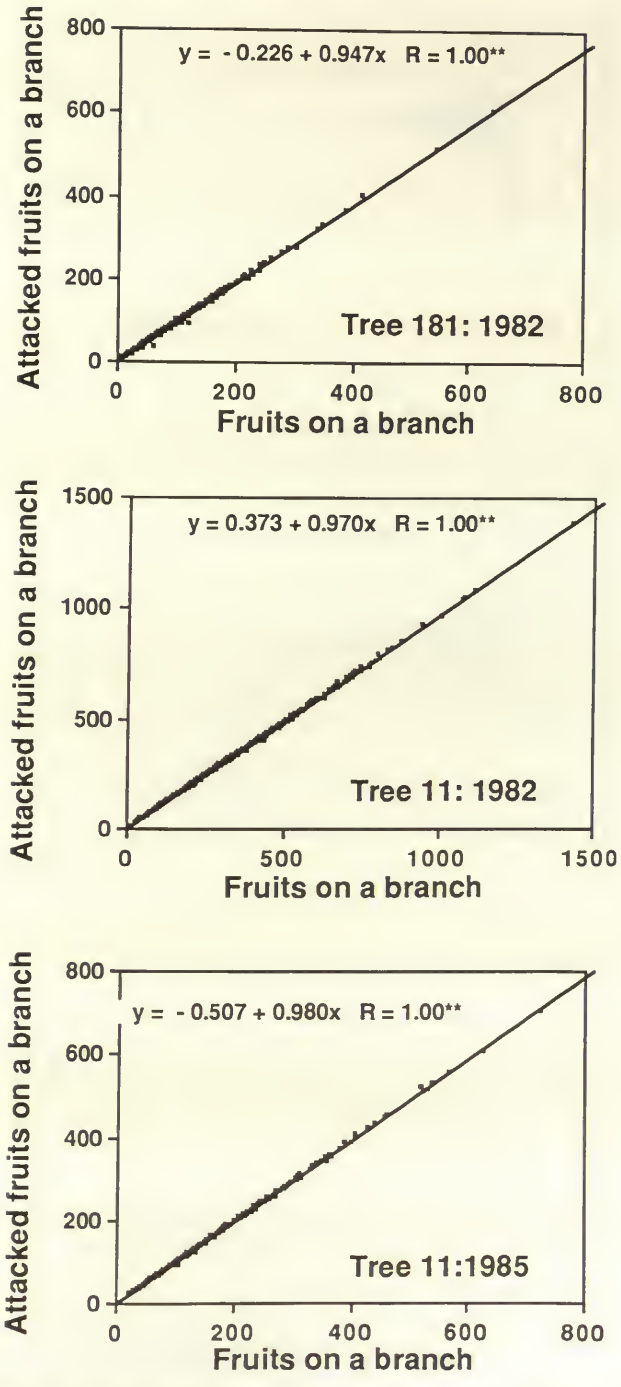


FIGURE 13. Relationship of number of attacked fruits on a branch to the number of fruits on a branch within three large samples of *Ateleia herbert-smithii* (see Appendices V–VII for sample sizes). Same locale as in Figure 1.

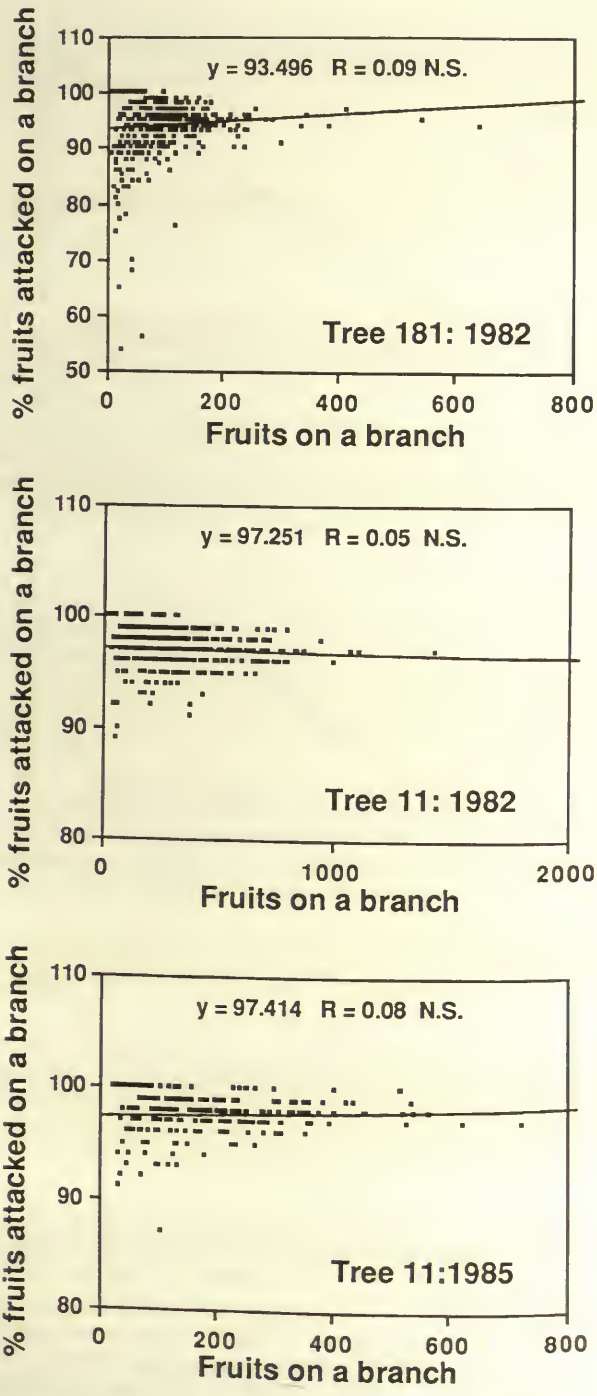


FIGURE 14. Relationship of percent fruits attacked on a branch to the number of fruits on a branch. Same tree and locale as in Figure 13.

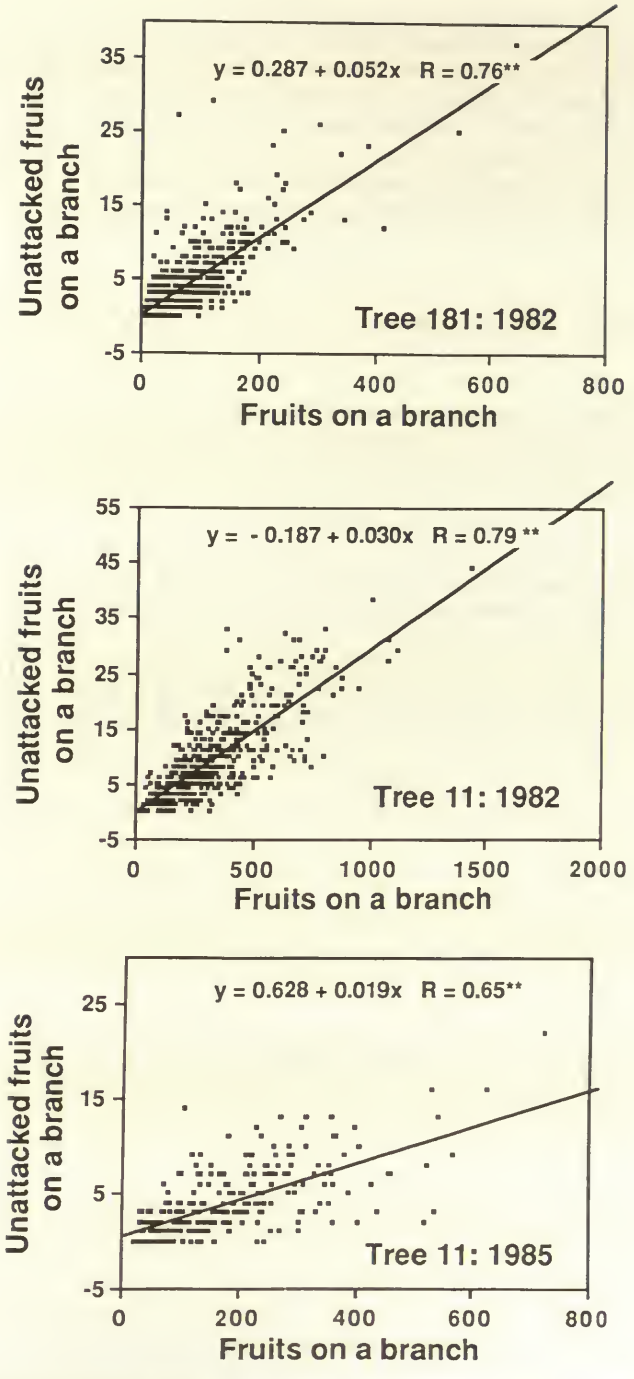


FIGURE 15. Relationship of the number of unattacked fruits on a branch to the number of fruits on a branch. Same tree and locale as in Figure 13.

b) A fruit may be overlooked by all ovipositing females. This might occur when an infructescence bears hundreds of fruits, and therefore the fruits are very tightly packed together (Fig. 6). Likewise, it might occur when there are only a few fruits in an infructescence, rendering the infructescence olfactorily inconspicuous in comparison with more normal infructescences in the same crown. These processes should result in there being slightly lower percentages of attacked fruits in very small and very large infructescences. There are more infructescences with slightly reduced percentages of attacked fruits among the small infructescences in Figure 14 than among the others, suggesting that small infructescences might be occasionally overlooked or bypassed (in the presence of a large number of other potential oviposition sites). Likewise, very young trees with only a few fruits in their crops are the source of exceptionally high seed survival within a crown (e.g., Fig. 8a, b). Interestingly, this relationship occurs even when the young tree is only a few tens of meters from a large adult bearing a large fruit crop.

c) In crops with greater than 95% seed predation, surviving seeds are commonly in fruits where some other insect has eaten small amounts out of the thin bladelike margin of the fruit wall. This implies that the adult weevils avoid ovipositing in fruits damaged in this manner. If there truly is avoidance, it may be that the herbivore damage mimics the cues that a weevil uses to avoid ovipositing in a fruit in which oviposition has previously occurred (if this is indeed why only one weevil develops per fruit). Response to such signs seems likely in the case where only one larva can develop in a fruit and there is an abundance of fruits in which to oviposit. Alternatively, the weevil may simply avoid ovipositing in damaged fruits (the selection for such behavior might have been that such fruits have a reasonable probability of containing no seed or of being aborted by the parent on a later date).

d) I have seen no sign of egg parasitoids (which also would leave some attacked fruits without larvae), but they could occur. The weevil larvae are attacked by hymenopterous parasitoids, but the wasp larvae do not kill the weevil larvae until after the seeds have been killed (see below).

2. *In 2-seeded fruits*

Two-seeded fruits (Table 5) are an obvious source of surviving seeds in some crowns (e.g., Fig. 16). There is no suggestion that a pair of weevils can co-occur in a two-seeded fruit. When there are two seeds in a fruit, the weevil larva usually kills only one of them (Fig. 7). This means that attacked 2-seeded fruits have a much higher chance of producing one surviving seed than do 1-seeded fruits. The total percent seed survival that is due to 2-seeded fruits varies from 0–80% among trees (Fig. 16; Table 6; and see tree 20 in 1985 in Appendix I). This percentage is a function of two different processes: the percent of the crop that is 2-seeded (Table 5) and the behavior of the larvae in the fruits. First, most trees have so few 2-seeded fruits that even a high percent of survival due to 2-seeded fruits makes relatively little contribution to the total percent of seed survival. However, if attack is intense, even a few survivors contributed by 2-seeded fruits may be a large proportion of the surviving seeds (e.g., many trees in 1985 had less than 5% 2-seeded fruits in the crop, but 20–50% of the seed survival was due to escape of one of the seeds in 2-seeded fruits: Appendix I; see also Fig. 8e).

Second, in some crowns in some years the single larva frequently mined into both seeds, thereby eliminating increased survival due to 2-seeded fruits; the cause of variation in whether 1 or 2 seeds are killed is not obvious. The outcome of these two different processes acting in concert is that when a small percent of a tree's fruit crop is 2-seeded, it is not possible to predict either the percent of surviving seeds or what percent of the surviving seeds will be in 2-seeded fruits. However, when a tree's fruit crop has many 2-seeded fruits, the percent of seeds surviving in the crop will be somewhat greater than the population average, and a high percent of the surviving seeds will be in

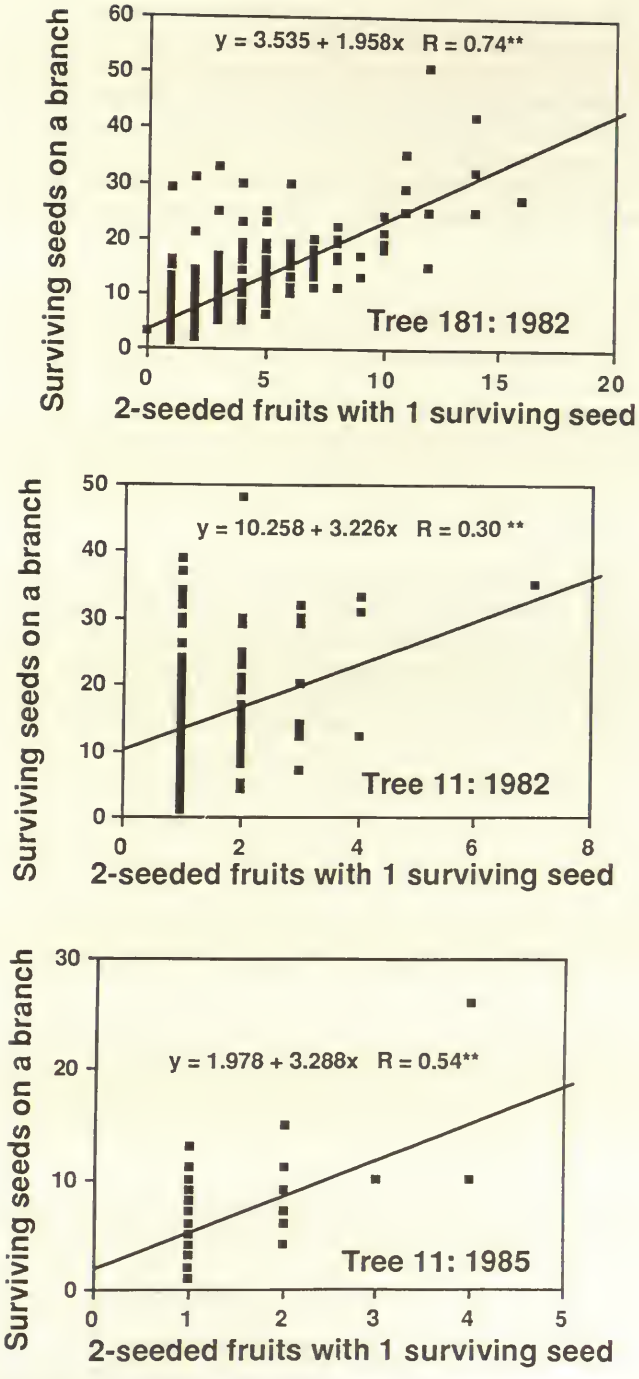


FIGURE 16. Relationship of the number of surviving seeds on a branch to the number of two-seeded fruits with one surviving seed. Same tree and locale as in Figure 13.

2-seeded fruits (Fig. 16; Appendix I). The large number of trees sampled in 1982 allows an overall comparison between crowns; there is a barely significant negative regression of the percent of seeds killed in all of a crop against the percent of the fruits in that crop that are 2-seeded (Fig. 8f).

In general, 2-seeded fruits are not common enough to seriously alter the pattern of seed predation by weevils that is obtained by considering the fruits attacked (Figs. 13–15) and that obtained by considering the actual number of seeds killed (Figs. 17–19). However, there is a slight increase in slope in Figure 19 as compared with Figure 15. This increase is due to the presence of 2-seeded fruits, which results in some attacked fruits still bearing a viable seed. Overall, the outstanding relationship is that as the number of seeds in an infructescence increases, the number of surviving seeds in that infructescence increases (Fig. 19; Table 7). It is also true that as the number of 2-seeded fruits in an infructescence increases, the number of surviving seeds on that branch increases (Fig. 16; and see 1982, 1984, and 1985 in Table 7). This is partly because the overall number of surviving seeds is so low that the proportional contribution from 2-seeded fruits is very high, and partly because the number of 2-seeded fruits on an infructescence increases as the total number of fruits on that infructescence increases. Note, however, that the larger absolute number of surviving seeds in 1-seeded fruits per branch increases as the number of fruits on that branch increases.

3. Seed predator satiation

If an increase in weevils or weevil oviposition capacity within a crown or tree population leads to an increase in the percent of seed mortality within a crown or tree population, seed predator satiation is occurring. However, even when there are so many weevils that satiation is no longer occurring, the bigger the seed crop borne by an *Ateleia herbert-smithii* crown, the larger will be the absolute number of surviving seeds that it generates (e.g., Fig. 8d). The intertree variation in absolute number of surviving seeds is very great (Appendix I; Fig. 8d) though not nearly as great as is the intertree variation in total crop size. A small fruit crop and an enormous fruit crop may produce the same (small) number of viable seeds.

a) *Satiation within a tree's crown.* Within any given year, the percent of fruit attack in a tree's crop was not correlated with fruit crop size except in 1982 (nonparametric test, Table 7). I suspect that in 1982 there were so few weevils that the massive population-wide fruiting resulted in some seed predator satiation in certain tree crowns. However, even in 1982 the regressions of the percent of fruits attacked or the percent of seeds killed on the estimated fruit crop size (Fig. 8a, b) were significant. The very largest crops recorded (1–2 million fruits) suffered about the same percent of seed predation within the crown as did more normal crops. Since the percent of seed predation within a crown is uniformly high (e.g., Fig. 8a, b), it is likely that either a tree usually attracts weevils in proportion to the number of fruits that it bears (over a wide range of crop sizes) or large numbers of weevils arrive at all trees. If the latter is the case, many of these must then leave the smaller crops in search of larger crops (with the consequence of an accumulation of weevils on a tree in proportion to the size of its crop).

There are cases in which the ovipositing females that arrive at a tree's crown appear to collectively lack sufficient eggs to be able to oviposit on all the fruits (i.e., intracrown seed predator satiation has occurred). This is the most likely primary cause of cases of seed mortality less than about 90% (e.g., Appendix I). For example, the fruit crop of tree 130 was of two age groups in 1984 (Appendix I). Its older fruits (from the first flowers) matured in synchrony with the majority of the *Ateleia herbert-smithii* population, and 99% of those seeds were killed by weevils. Its younger fruits (from a late pulse of flowering) matured long after most weevils had (presumably) laid their eggs; the younger fruits suffered only 58% seed loss. Again, tree 296 in 1982 flowered at the end of the general flowering period in a year in which virtually all female trees made full-sized crops; its fruits were therefore maturing at the time when the weevil population should have nearly exhausted its

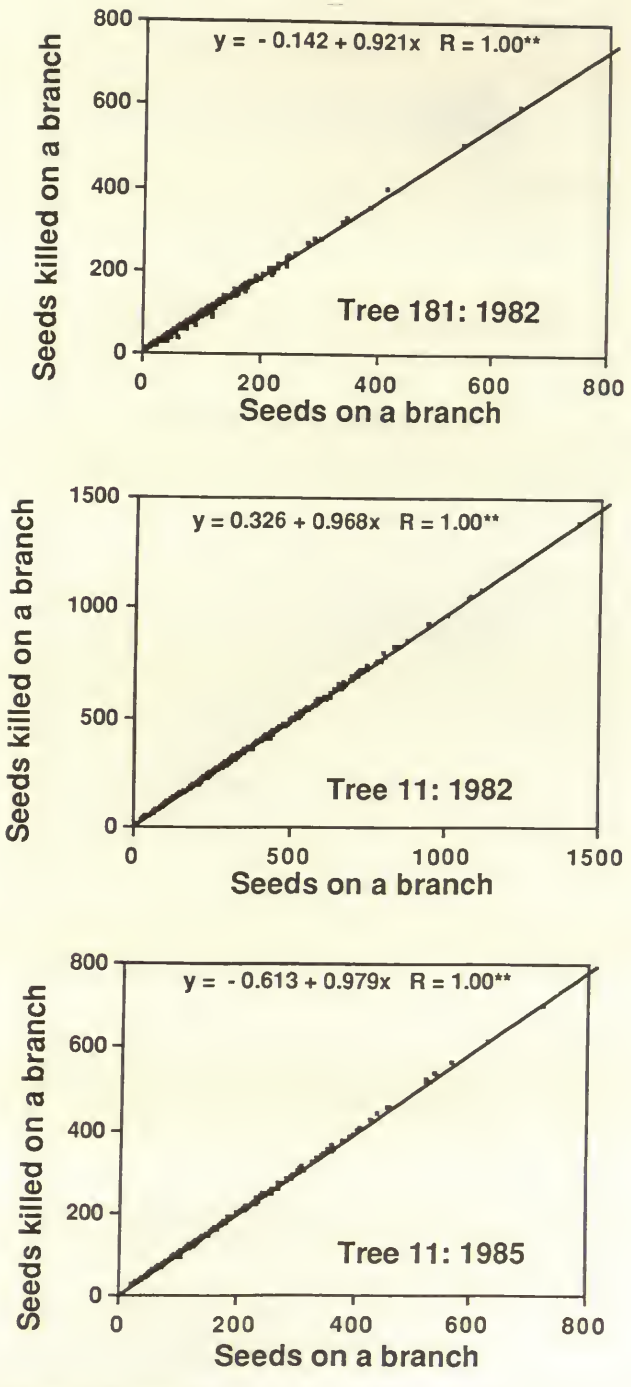


FIGURE 17. Relationship of the number of seeds killed on a branch to the number of seeds on a branch. Same tree and locale as in Figure 13.

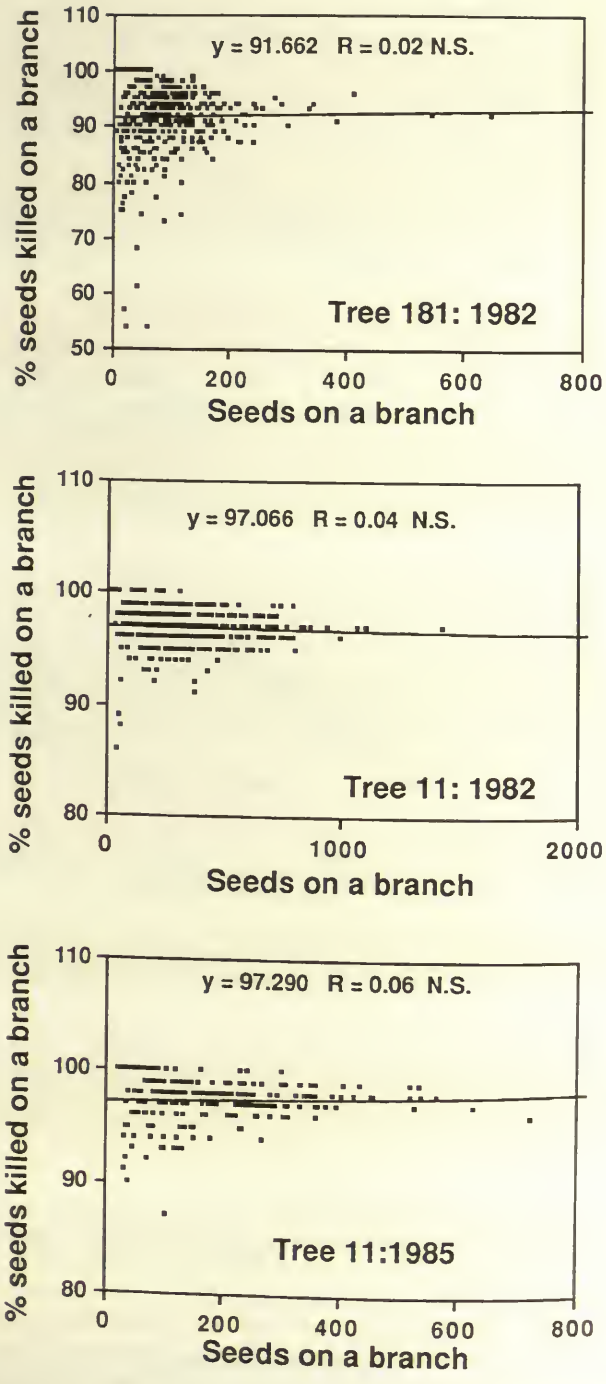


FIGURE 18. Relationship of the percent of the seeds killed on a branch to the number of seeds on a branch. Same tree and locale as in Figure 13.

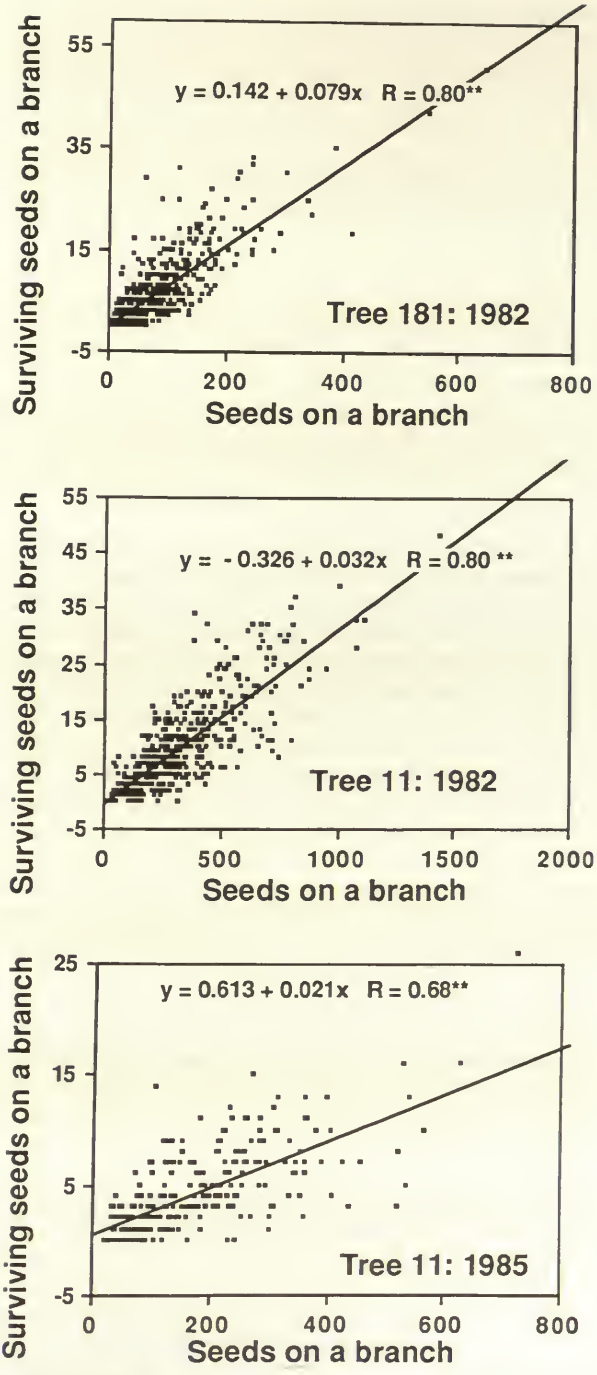


FIGURE 19. Relationship of the number of surviving seeds on a branch to the number of seeds on a branch. Same tree and locale as in Figure 13.

TABLE 7. Results of Spearman rank correlation tests of *Ateleia herbert-smithii* fruit and seed crop variables in Santa Rosa National Park from 1974 to 1985.

Figure number and subject	1974	1976	1978	1980	1982	1984	1985
1. % fruits attacked × fruit crop size	ns	ns	ns	ns	***	ns	ns
2. % seeds killed × fruit crop size	ns	ns	ns	ns	***	ns	ns
3. Number of seeds surviving × number of fruits attacked	***	***	ns	ns	ns	ns	***
4. % seeds killed × % fruits two-seeded	ns	ns	NA	ns	***	ns	***
5. Number of seeds surviving × number of fruits two-seeded	ns	ns	NA	ns	**	***	***
6. Number of seeds surviving × seed crop size	***	***	***	***	***	***	***

oviposition capacity, and it suffered only 25–31% seed predation. In this case, both within-crown and within-population seed-predator satiation occurred.

There is a second cause of seed survival that appears to be within-crown pre-dispersal seed predator satiation but is not necessarily so. Very young trees often have very small fruit crops. These small crops, often containing only a few infructescences, sometimes have a quite low percent of seed mortality (e.g., Fig. 8a, b, and especially tree 184 in 1982). This mortality is, however, of a quite distinctive kind. Most of the surviving seeds are in one or two of the few infructescences, strongly implying that those infructescences were simply missed or located infrequently by weevils (e.g., seed survival due to errors), or the weevils that arrived had few eggs left to lay. Strictly speaking, only the latter case is seed predator satiation at the level of the individual fruit crop. Large trees bearing exceptionally small seed crops show no sign of diminished levels of seed predation. I suspect that the vegetative tree itself provides the odor cue to the weevils, and therefore many arrive irrespective of whether the fruit crop is large or small.

b) *Within the population.* The percent of attack by the weevil has been generally increasing over the past 11 years at Santa Rosa (Tables 3, 4). That is to say, the percent of seed predator satiation across the population as a whole has been declining at least through 1982. The 1984 and 1985 percent of seed survival may be as great as can occur once the weevil population has reached such a high relative level that it cannot be satiated. As mentioned earlier, the 1985 record percent of seed predation levels may represent the combined effects of a relatively small seed crop for the population as a whole and only a single year between crops. These two things suggest that an excessive number of weevils probably arrived at a relatively small number of fruits. When all trees in the population suffer greater than 92% seed mortality, as occurred in 1985, there is essentially no population-wide predator satiation, and almost all seed escape appears to be due to errors by the weevils, 2-seeded fruits, etc.

The few trees that partly escape the weevil may produce as many viable seeds as will tens to hundreds of other fruiting trees combined (Table 2). The overall number of surviving seeds per tree has a distribution that does not seriously deviate from normal, but the few individuals that partly escape make a contribution to the population-level seed production that is many standard deviations from the mean. For example, during this study, the single tree with the largest number of surviving seeds in its year made up the following percentages of the total surviving seed crop censused in each year (Table 2): 16, 35, 44, 29, 8, 40, and 29 (note, however, that this percent is a function of how many trees are contained in the sample). If a large seed crop in a particular tree suffers a low percent of seed predation, it is quite possible for that particular tree to produce as many seeds as do all the

combined female members of the population within several kilometers of that tree. On the other hand, since the fruits are wind-dispersed, the few trees with high seed survival do not encroach on the seed shadows generated by other trees nearly as much as would be the case were *Ateleia herbert-smithii* dispersed by animals.

The Nicaraguan *Ateleia herbert-smithii* population in the region of El Bordo de las Tinajas suffers only trivial pre-dispersal seed predation by insects (C. Hughes and B. Styles, pers. comm.). Since the population grows in a severely disturbed area that contains only fragments of badly disturbed secondary successional deciduous forest that are widely scattered over much pasture and cropland, the low percent of seed predation could well be due to a lack of adequate sites in which the adult weevils can pass the time between fruit crops. Depression or elimination of insect seed predator density in agriculturized habitats is commonplace to the south of Santa Rosa National Park in Guanacaste Province (unpublished collection records). It even occurs on the boundary between SRNP and Hacienda Rosa Maria, where the few *A. herbert-smithii* that have been left in tiny patches of forest among the extensive cotton and sorghum fields suffer little pre-dispersal seed predation (e.g., tree 402, 1984). Both habitat destruction and agrochemicals are probably responsible for depression of seed predator density in Guanacaste. However, it is possible that the pristine original Nicaraguan population initially lacked a substantial degree of pre-dispersal seed predation by the weevil, but that is now unknowable.

3. *Parasitoids.* I have reared hundreds of thousands of adult weevils from fruits that were collected in the crowns of *Ateleia herbert-smithii* trees in Santa Rosa's forests and abandoned pasture margins. The number of hymenopterous parasitoids emerging from the fruits along with the weevils show that weevil larvae suffer an attack frequency of about 0–2%. These tiny parasitoids have not yet been identified, but there are at least six species, and they appear to be species I have reared from other species of weevils and bruchid beetles whose larvae develop in Santa Rosa flower buds and seeds in maturing fruits. The wasp species reared from *Apion johnschmitti* are probably maintained most of the time by successive generations in prey species other than this weevil. However, they move onto the huge food resource of weevils that is produced when there is an *A. herbert-smithii* crop, and during that year enormous numbers of wasps are produced by the crop (more than by any other species of woody plant in the habitat). The *A. herbert-smithii* crop probably generates more parasitoids than do all the other hosts combined.

The seeds that are attacked by parasitized weevil larvae are killed and as thoroughly consumed as are those attacked by unparasitized weevil larvae. Since there is only one weevil generation per *A. herbert-smithii* crop, the weevil mortality due to these hymenopterous parasitoids within a crown and year is irrelevant to the biology of *A. herbert-smithii* in that year. Furthermore, its intensity is determined by the numbers of parasitoids generated by the non-*Apion johnschmitti* hosts in the previous year. However, these parasitoids may increase survival of *Ateleia herbert-smithii* seeds in the population as a whole. For example, if parasitoids reduce the number of weevils produced overall by the *A. herbert-smithii* population in a given year, somewhat fewer weevils may be available to oviposit in the subsequent fruit crop. Even a slight reduction in the percent attack in the subsequent crop can be highly significant if pre-dispersal seed predation is very high (as it is in *A. herbert-smithii*); a reduction in percent of seed predation from 99% to 98% represents a doubling in the size of the crop of viable seeds. However, it is also probable that a few percent of reduction in the weevil population by parasitoids will not lead to an observable reduction in the percent of seed predation, when that percent of seed predation is set by errors rather than by seed predator satiation.

PRE-DISPERSAL SEED PREDATION BY OTHER ORGANISMS

No other species of weevil (or other seed predator beetle) has ever been reared from *Ateleia herbert-smithii* in Santa Rosa (or elsewhere). However, the Nicaraguan population at El Bordo Las

Tinajas has not been examined for weevils or other seed predators in general; the same lack of attention applies to other *Ateleia* spp. populations.

A minute fraction of the *A. herbert-smithii* fruit crop at Santa Rosa is also attacked by the larvae of a phyticine pyralid moth. The larva mines inside the fruit and consumes the seed. A larva is normally restricted to one seed and starts feeding on it after the seed is full-sized. However, in the laboratory, a larva may move from fruit to fruit if the fruits with intact seeds are clumped together in a container. This moth usually attacks fewer than 0.01% of the fruits on a tree, but the larvae are found exclusively in the few seeds that have been missed by the weevils (some of which are in 2-seeded fruits). The result is that they may kill as many as 5% of the surviving seeds in some crops, although the usual amount is less than 1%. Damage by this pyralid moth was not systematically recorded and is not included in the mortality figures in Appendix I (e.g., if a tree is recorded as having 91 surviving seeds, three of them might have been in fact killed by the larvae of the pyralid).

In a few cases an unidentified orthopteran eats the seed-containing centers of fruits. I do not know if it feeds on all kinds of *Ateleia herbert-smithii* fruits or only those with intact seeds. Unidentified parrots opened a few green fruits with intact seeds in the 1982 fruit crop, but this damage, like that of the orthopteran, was trivial.

POST-DISPERSAL SEED PREDATION

No animal regularily attacks or moves *Ateleia herbert-smithii* fruits (or dormant seeds) once they have been dispersed. In a single unique case, I found that a small rodent had removed the seeds from several hundred fruits below tree 11 in 1978. The response of animals to newly germinated *A. herbert-smithii* seeds has not been examined.

The habitat into which *Ateleia herbert-smithii* seeds fall is thoroughly foraged by a small rodent seed predator, *Liomus salvini* (Heteromyidae). When *A. herbert-smithii* fruits or clean seeds are placed at a *L. salvini* feeding station, they are left undisturbed. When the seeds are given to an *L. salvini* in the laboratory, it chooses to die of starvation rather than eat them. 2,4-methanoglutamic acid and 2,4-methanoproline extracted from Santa Rosa *A. herbert-smithii* seed (provided by R. Nash, and see Bell et al., 1980) was mixed into laboratory chow at 0.5% and 1.0%, respectively (the concentrations at which they occur in *A. herbert-smithii* seed); *L. salvini* in the laboratory at Santa Rosa (five adult males and five adult females, August 1984) ate the adulturated chow with gusto and grew fat on it. These two uncommon amino acids are clearly not the major cause of rejection of *A. herbert-smithii* seeds by *L. salvini*. Ground up *A. herbert-smithii* seeds did not show any protease inhibitor activity nor show a hemagglutinating response to rabbit or type B human blood (Janzen et al., 1986); however, these results certainly do not exclude the possibility that *A. herbert-smithii* seeds contain a toxic protein, since some hemagglutinins (lectins) are toxic to *L. salvini* (Janzen, 1981), and many more kinds of blood would have to be screened against *A. herbert-smithii* seeds before it could be stated that they contain absolutely no lectins. So the rejection of *A. herbert-smithii* seeds by *L. salvini* remains very much a mystery.

V. Growth

As described earlier, male and female *Ateleia herbert-smithii* have somewhat different life forms (Figs. 1, 2). Trees of both sexes may attain an age of at least 200 years. This figure is based on counting the conspicuous annual rings in the sapwood; *A. herbert-smithii*, like numerous other species of Santa Rosa trees, have conspicuous annual rings that are associated with the cessation of growth during the six-month dry season. Additionally, tree ages have been estimated by extrapolating from diameter increments (Appendices I–IV). Trees of 200 years of age are 0.8–1.3 m dbh and usually grow in small old pastures where they have received full sunlight and other trees have

not grown up because annual grass fires have kept them out. These fires have, however, not killed the *A. herbert-smithii* for two reasons. First, the area directly below a large old *A. herbert-smithii* is often choked with woody shrubs that eliminate grass by their shade, and therefore protect the lower trunk and limbs of the *A. herbert-smithii* from grass fires. Second, grazing by cattle helps the *A. herbert-smithii* by minimizing the amount of grass fuel; following removal of cattle from Santa Rosa in 1978–1979, many large *A. herbert-smithii* in pastures or on pasture edges were killed by the very intense fires in subsequent years. However, it is not clear why truly old *A. herbert-smithii* that are protected from wind and fire die in Santa Rosa.

A more common size for reproductive adults in forest is 30–70 cm dbh (i.e., 50–150 years old). However, trees greater than about 40 cm dbh on shallow soils are subject to being uprooted by early dry season (January) winds. I have encountered at least 50 recently dead *A. herbert-smithii* in the past 12 years, and all but one death was due to wind-throw or to trunk breakage following fire damage to the lower trunk. Wind-thrown trees were usually uprooted following the breakage of major surface roots 1–2 m from the bole and during the period of intense winds in January. The fire-killed trees were growing on a pasture–forest interface, and a strong downwind fire moved into the forest from the pasture. The cambial layer near ground level was killed by the heat, leaving a patch of dying tissue. The next fire, in some later year, then had access to the dead core area and burned through the base of the tree; the subsequent (third) fire to pass by consumed the newly fallen tree.

A large *Ateleia herbert-smithii* that is subjected to only 1 or 2 ground-level fires often develops a hollow core, and this core is a roosting and nesting area for animals. Roots often grow from the inner lower walls of the tree directly into this (presumably) nutrient-rich dung heap (Fig. 20b, c). Likewise, if the hollow core extends upward through the bole, these internal roots grow upwards to moist pockets caused by rain oozing in the core from places where the core hollow breaks through the bole (usually in the forks of large branches).

During the period from about 2–3 years of age to senescence, healthy *Ateleia herbert-smithii* with insolated crowns add an average of about 0.5 cm to their dbh per year. Seventy-eight healthy adult female trees were observed for two years, and their annual dbh increment was an average of 0.5 cm (s.d. = 0.5, range 0–2.9 cm; initial diameters 6.1–71.4 cm, average 32.4 cm, s.d. = 1.5) (Appendix III). Nine males observed during the same period had an average diameter increment of 0.4 cm per year (s.d. = 0.2, range 0.1–0.7 cm; initial diameters 31–63 cm, average 48.9 cm, s.d. = 9.2) (Appendix II). Sixteen healthy adult females were observed for eight years, and their average diameter increment was 0.5 cm (s.d. = 0.4, range 0.1–1.5 cm, average initial diameters 10.4–87.1 cm, average 39.5 cm, s.d. = 20.9) (Appendix IV). None of these three increment rates are significantly different from each other (Spearman rank correlation test). In all three sets of wood increment records, the slope of the regression of annual diameter increment against the initial diameter of the trunk was not significantly different from zero, and the three correlation coefficients were also not significant. All these statements were also true of the subset of the female trees that were watched for two years and that fell within the diameter range of the male trees in Appendix II. The very large *A. herbert-smithii* with a dbh of greater than 1–1.5 m have highly fluted trunks (Fig. 20a, b), and diameter increment measurements are difficult to record and interpret. Younger trees sometimes have less severely fluted trunks.

Ateleia herbert-smithii are as tall or taller than are any of the trees with which they grow. Even though they sprout on bare soil (or in herbaceous growth) in the earliest stages of succession, the adults are not shaded out at a later stage. The slower-growing evergreen species of later succession that form a substantial part of the canopy of pristine forest (e.g., *Manilkara, Hymenaea, Mastichodendron, Swartzia, Swietenia, Quercus*) do not grow taller than do *A. herbert-smithii* adults at Santa Rosa. The same applies to the many species of deciduous trees that co-occur with *A. herbert-smithii* both in the early stages of succession and later. However, these large and tall adult *A. herbert-smithii* produce virtually no surviving seedlings and saplings in the forest understory, even when it is a vegetation that is very deciduous in the dry season. As the old adults die through

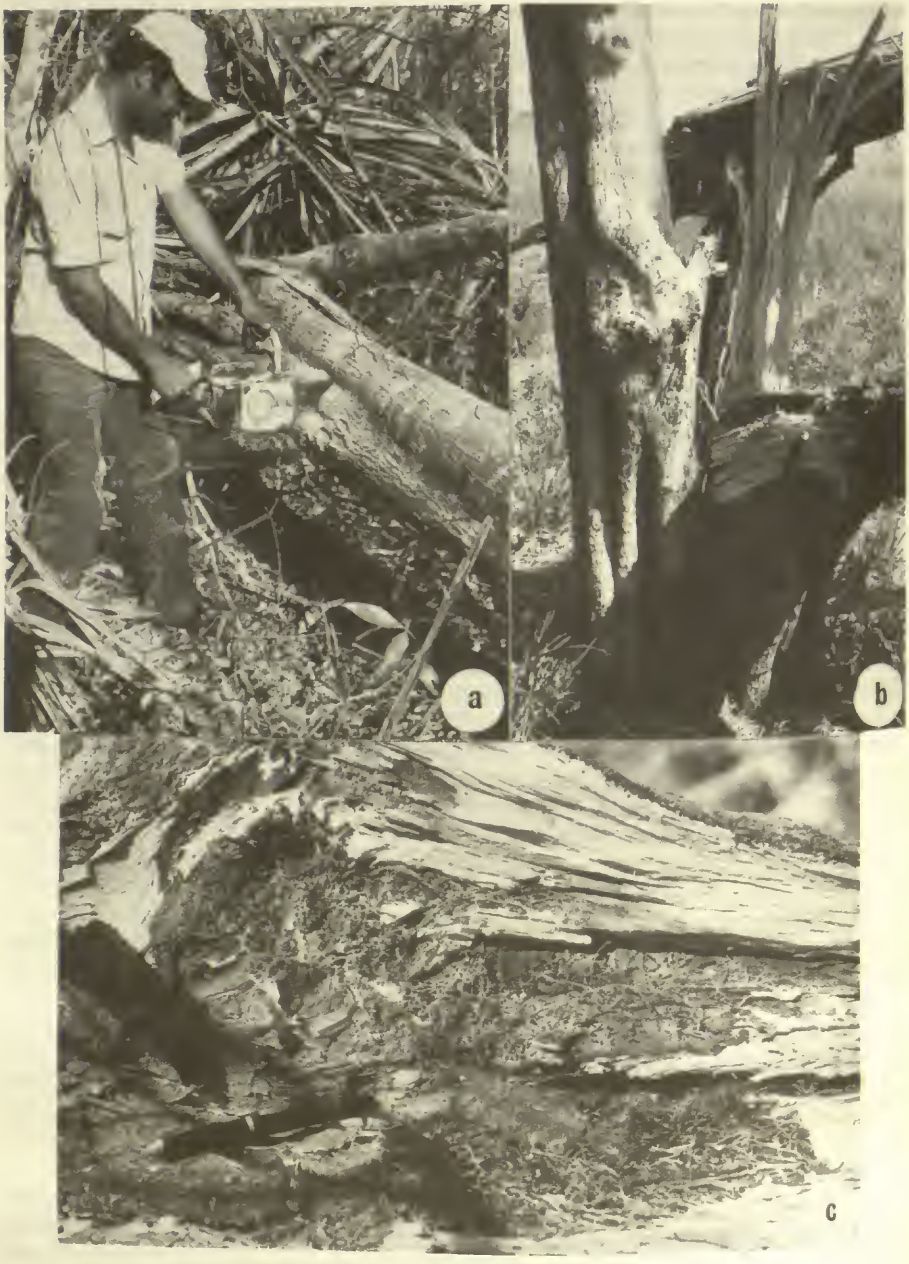


FIGURE 20.—a. Forty-year-old *Ateleia herbert-smithii* immediately after being uprooted by January winds in same locale as Figure 1.—b. Fifty-year-old forked *A. herbert-smithii* split by January winds 3 km south of Figure 1 locale; the split at the fork was made likely by a rotten core that got started in an old fire scar. At the center of the photograph is a root extending from the left-hand standing portion of the tree into the downed portion.—c. Center of downed trunk in b above, filled with rotted wood and guano, and thoroughly penetrated by adventitious roots of *A. herbert-smithii* growing out of the sides of the cavity in the trunk.

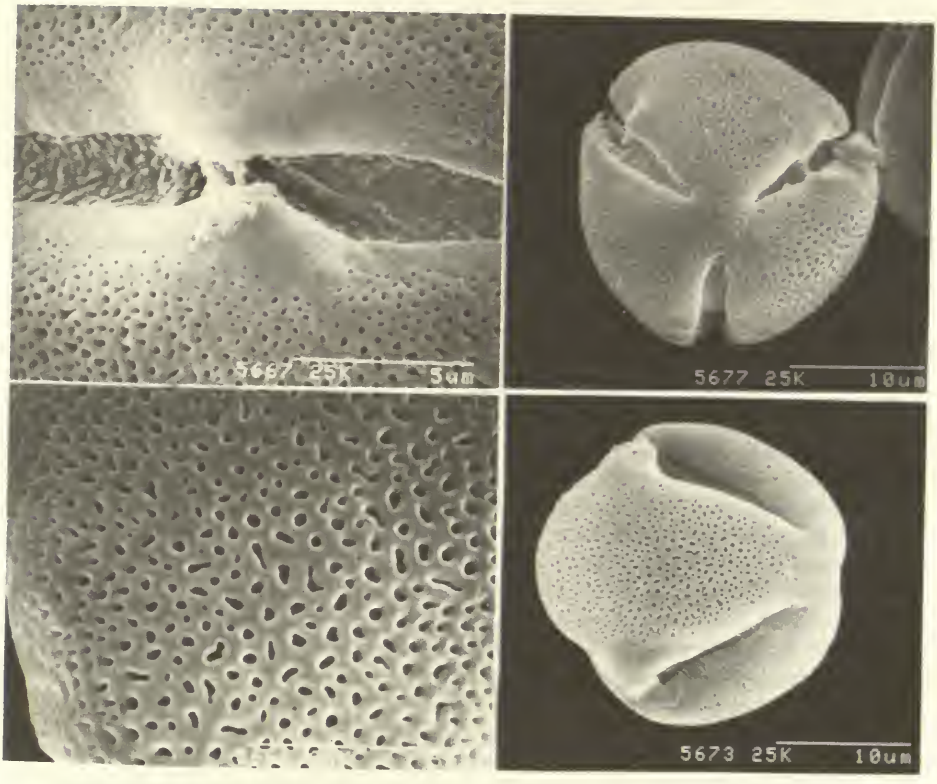


FIGURE 21.—a. Polar view of a representative tricolporate *Ateleia herbert-smithii* pollen grain.—b. Lateral view of a above.—c, d. Fine detail of a and b Specimens (DHJ 11511) from Santa Rosa National Park, prepared by C. Batchelor and I. K. Ferguson (1986).

being wind-thrown, they are replaced by other species, except in a very restricted set of habitats. It appears that *A. herbert-smithii* was originally a common deciduous member of the disturbed sites and tree falls of relatively evergreen oak forest *(Quercus oleoides)* and perhaps a rare member of semi-evergreen to nearly purely deciduous forest in a few very rocky sites in the eastern end of Santa Rosa. When such a mosaic of oak and semideciduous forest is patchily and incompletely agriculturized (selectively logged or patchily burned, pastured, and browsed), seedling and sapling survival conditions for *A. herbert-smithii* are greatly improved. It is likely that the tree has become much more common during the last 300 years and even occupies some forest succession on very moist and very dry sites that were probably not originally occupied by it. It becomes the most abundant where pasture or road edges are cleared of forest within about 100 m of large adult *A. herbert-smithii* and then allowed to return to woody vegetation through prevention of fires. In such sites, it may form monospecific stands of 10–30 m diameter. As such forest again returns to its original state, *A. herbert-smithii* again becomes a rarer and more habitat-specific tree. This is currently happening to most of its population in Santa Rosa, where it was and is a rapid and frequent colonizer of pastures that were abandoned when the park was formed in 1968–1972. It is not replacing itself in most sites in 50- to 200-year-old secondary successional forest.

Ateleia herbert-smithii usually does not sprout from the bole, roots, or branches when wind-thrown or burned. However, a young to middle-aged tree that has been cut off near ground level may produce sucker shoots if the site is insolated. Saplings with their crowns cut or smashed out of

them produce straight and vertical sucker shoots. Saplings with their tops burned off of them by grass fires only very rarely make sucker sprouts; in general, fires eliminate young stands of *A. herbert-smithii*. Branches cut out of crowns and stuck into the ground usually die, but they sometimes root, especially if this is done at the beginning of the rainy season.

VI. Herbivory

Very few foliage-eating herbivores feed on *Ateleia herbert-smithii*. In the 1977 rainy season, the Santa Rosa adults were severely defoliated by the green caterpillars (1–2 cm long) of a medium-sized noctuid moth (*Parachabora abydas* H.S.). However, in each year since, the amount of leaf damage by this and all other herbivores has been only 0–2% of the total leaf area. *Parachabora abydas* caterpillars are present every year during the rainy season, but at very low density. Its caterpillars feed only on *A. herbert-smithii* at Santa Rosa (results from an eight-year ongoing survey of caterpillar host plants at Santa Rosa). To date, *P. abydas* has been the only species of caterpillar found feeding on *A. herbert-smithii* leaves. However, large turds of a caterpillar in the family Saturniidae were found once under an isolated *A. herbert-smithii*, and therefore at least one other species of caterpillar can eat its foliage.

The *Ateleia herbert-smithii* that were defoliated by caterpillars in 1977 made a new leaf crop within 1–2 months. During an artificial defoliation experiment with tree 11 (female) on 10 August 1984, all of the leaves subtending the 30 bud-bearing terminal inflorescences on eight large branches were carefully cut off by cutting the petiole without tearing the leaf connection to the branch. By 3 November 1984, these branches had aborted all inflorescences and produced a whorl of fully expanded but smaller leaves on an axillary shoot originating from the axil of one of the old cut petiole bases. The same experiment performed on tree 141 (male) resulted in retention of the inflorescences, and no new leaf whorl was produced. However, some of the inflorescence branchlets did not fully expand, and many individual florets were shed as buds. Simultaneously, the above experiment was modified such that only one side of a forked branch end was defoliated, and it was performed on the same individuals of both sexes. The defoliated branches retained their inflorescence and flowers, and the defoliated branches on the female tree even set some fruits.

No indication of browsing of *Ateleia herbert-smithii* foliage or twigs by deer or other vertebrates has been seen in Santa Rosa. *Ateleia herbert-smithii* trees are ignored even in very heavily browsed/grazed cattle pastures. An adult captive Baird's tapir *(Tapirus bairdii)* absolutely rejected foliage without even tasting it; this individual animal came from a part of Costa Rica where *A. herbert-smithii* does not occur (Janzen, 1982b), and therefore the rejection response could not have been learned.

The crushed leaves and bark (which is green just under the thin gray epidermis) of *Ateleia herbert-smithii* have a distinctive and very strong garliclike odor, suggesting an exceptionally high concentration of a volatile and potentially defensive chemical (and Rudd [1968] noted that a common name of *A. gummifera* is *balsamo hediondo* [''stinking balsam'']). On the other hand, *A. herbert-smithii* leaves have quite low amounts of total phenolics and condensed tannins (Janzen & Waterman, 1984); this apparent paucity of digestion-inhibiting chemicals and prominence of a (small) volatile molecule, taken in concert with the fact that the noctuid *Parachabora abydas* is monophagous, suggests that *A. herbert-smithii* will not be fed on by relatively polyphagous herbivores.

Living *A. herbert-smithii* trunks that are wounded by the larvae of wood-boring beetles produce copious quantities of brownish gum or resin.

The roots of the Santa Rosa *Ateleia herbert-smithii* are conspicuously nodulated. C. Hughes (pers. comm.) reports that the Nicaraguan *A. herbert-smithii* are also nodulated; *Rhizobium* has been isolated from the nodules (Halliday & Somasegaran, 1984).

The sap and heartwood of dead trees is rapidly decomposed by termites, wood-boring beetles,

and fungi; there is no obvious difference in hardness or chemical composition between the cream-colored heartwood and sapwood. Freshly cut or broken *Ateleia herbert-smithii* branches may be located within a few hours as an oviposition site by a yellow and black cerambycid beetle (*Placosternus crinicornis* Chev.).

VII. Discussion

Ateleia herbert-smithii is the only legume in the world that is known to be wind-pollinated. However, I strongly suspect that the approximately 17 other species of *Ateleia* (Dillon, 1980; Rudd, 1968) are also wind-pollinated. Their flowers look just like those of *A. herbert-smithii* (e.g., see drawings of *A. arsenii* in Polhill [1981: 219], and descriptions in Rudd [1968]). I suspect that the drawings showing well-developed *Ateleia* anthers positioned next to a comparatively huge and fully exposed discoid stigmatic surface should be viewed as the product of artistic license and a temptation to draw shriveled stamens or stigmas in a fully expanded manner. I also suspect that all *Ateleia* are dioecious, just as is *A. herbert-smithii;* those descriptions that mention *Ateleia* stigmas describe them as discoid, sessile, and/or peltate. It is likely that descriptions that ignore the stigma are based on material from a male tree (where the stigma is always severely shrunken) but it was simply assumed that the flowers were poorly preserved.

Discussion of the pollination systems of other potentially wind-pollinated legumes should be done by those who know them in the field. Arroyo (1981) suspected that "some Australian acacias might be anemophilous, at least facultatively"; however, P. Bernhardt (pers. comm.) is of the opinion that Australian acacias would be at best wind-pollinated only in the sense of being selfed by pollen falling from one flower to the next. C. Stirton (pers. comm.) suspects that *Colophospermum,* which grows in widespread monospecific stands in the Zambesi River basin, is a candidate for wind-pollination. Faegri & van der Pijl (1979) state that, "The corolla has been lost in [the Indian tree] *Hardwickia* (Caesalpiniaceae) and the blossom in anemophilus"; however this conclusion cannot be used without knowing its basis. Likewise, the *Ceratonia* and *Gleditsia* inflorescences figured on the back cover of Polhill & Raven (1981) are not only dioecious but look suspiciously like those of wind-pollinated plants.

There has been some question as to whether *Ateleia* should be placed within the papilionoid or caesalpinoid legumes (Rudd, 1968). While Polhill (1981: 213) firmly planted *Ateleia* in the Sophoreae (Papilionoideae), he noted that the Sophoreae "is a tribe of convenience between the Caesalpinioideae and the bulk of the Papilionoideae, sharply defined from neither." *Ateleia herbert-smithii* has a number of traits that are generally associated with caesalpinioids:

1. There are 8–10 stamens in most flowers, but flowers of *A. herbert-smithii* with 11–12 stamens are commonplace (and both C. Stirton and I have counted up to 13–14 in some); Rudd (1968: 391) noted that "instability of stamen number is a character more often found in caesalpinioid genera."

2. The peltate stigma of *Ateleia* (including *A. herbert-smithii*) would be "unique among the Faboideae, so far as I know, but it occurs in a few caesalpinioid genera such as *Arcoa, Bauhinia, Ceratonia,* and *Peltophorum*" (Rudd 1968: 391).

3. The strongly exserted stamens and radially symmetrical flower are strongly suggestive of the form of many caesalpinioid flowers (and see *Gleditsia* and *Ceratonia* photographs on the back cover of Polhill & Raven [1981]). This, along with (4) below, is why I earlier and naively placed it in the Caesalpinioideae (Janzen, 1978).

4. The embryo in the mature dormant seed is sandwiched between two layers of translucent and hard polysaccharide-rich endosperm. This trait is common in caesalpinioids (e.g., *Bauhinia, Cassia, Schizolobium, Delonix*), but I know of no papilionoids with this trait.

5. The single petal has the general shape of the stalked petals of many caesalpinioid flowers and does not resemble the petals of papilionoid flowers.

6. The severe reduction in petals (without even rudimentary ontogenetic initiation of petals) has

occurred on a number of occasions in caesalpinioids, but almost never in papilionoids (Tucker, 1989).

7. The floral organs are not initiated unidirectionally from the abaxial side, in contrast to the case with papilionoid legumes (Tucker, 1989).

8. In papilionoid legumes, the carpel usually arises along with the second whorl of stamens; in *A. herbert-smithii* it appears before the stamens do (Tucker 1989).

With the respect to the floral traits mentioned above, it is easy to view *Ateleia* as simply an ordinary caesalpinioid dish-shaped entomophilous flower that moved evolutionarily into wind pollination. For example, a single flowering branchlet of an *A. herbert-smithii* inflorescence superficially resembles an (insect-pollinated) *Mora megistosperma* (Caesalpinioideae) inflorescence. The loss of petals is the removal of an unrewarded cost and the removal of an obstruction to pollen launching and reception by the many small flowers. The peltate and sessile stigma is one of several possible solutions to the problem of how the size of the stigmatic surface can be increased in a habitat that is sufficiently dry to pose desiccation problems for a foliose stigma. On the other hand, it is also easy to imagine evolutionarily inventing an *Ateleia* flower from insect-pollinated Sophoreae such as *Acosmium, Myroxylon,* and *Lovanafia* (see figures in Polhill [1981: 219]).

But what about *Cyathostegia,* a close relative of *Ateleia*? Rudd (1968) felt most comfortable keeping *Cyathostegia* as a (perhaps monotypic) genus in a similarily uncertain position between the Caesalpinioideae and Papilionoideae. As noted earlier, R. Nash (pers. comm.) finds that the seeds (which Rudd viewed as morphologically very similar to those of *Ateleia*) contain the same uncommon amino acids as do those of *Ateleia* (Bell et al., 1980). While this will not help to understand its subfamily status, it is not difficult to view *Cyathostegia* as simply an *Ateleia* (or as having a common ancestor with *Ateleia*) with stamen multiplication in order to increase the amount of pollen produced. The pollinator of *Cyathostegia* is not known, but judging from the large number of strongly exposed anthers, it is either visited by a large hairy (or feathery) animal or wind-pollinated. In this context, it is interesting that it grows in xerophytic deciduous woodlands (Peru and Ecuador; Rudd, 1968), the same type of habitat occupied by *Ateleia* throughout its range (Rudd, 1968).

In view of the very central role given to floral parts in defining legume genera and higher groups, it is hard to avoid the conclusion that if more legumes were to have started down the road of wind-pollination, we would have considerably more groups as confusing as is *Ateleia.* Furthermore, genera such as *Ateleia* are prime suspects as the base for the evolution of major higher taxa. Assume that the evolutionary roots of *Ateleia* lie in some ordinary appearing legume. Through and during the evolution of wind-pollination coupled with dioecy, a rather unlegumelike flower has been evolutionarily invented. However, it is still similar enough to other legumes that its placement in the Leguminosae is incontestable. The argument lies in what subfamily or tribe it should be placed. Now, imagine that it were to find itself in a circumstance whereby there was intense realized selection for an insect-pollinated *Ateleia.* A variety of floral traits would be evolutionarily reinvented, but they would come from a highly modified legume flower. The evolutionary solutions to the standard problems in insect-pollination might well involve the production of traits viewed as decidedly nonlegume. The result would be a new family of plants, by what is hardly a profound evolutionary chain of events.

The extremely local distribution of *A. herbert-smithii* (and of the other *Ateleia;* Rudd, 1968) in Mesoamerica is probably intimately related to their dioecy and wind pollination. 1) One *A. herbert-smithii* does not a colonizer make. Since the sex ratio of adult plants is roughly 50:50, even two *A. herbert-smithii* will not be a colonizing population at least half of the time. This means that following any habitat change, the population has to expand and survive as a unit rather than as individuals making relatively long dispersal hops. 2) If there are to be equivalent levels of pollen donation and reception, the individuals of *A. herbert-smithii* must be much closer together in space and/or time than is generally the case for animal-pollinated tropical plants. It is striking in this context that *A. herbert-smithii* co-occurs with *Quercus oleoides,* the only other wind-pollinated tree in lowland Costa Rica that makes forests with single-species dominance. 3) Owing to the general

unidirectionality of wind flow (from the east) in Santa Rosa, male plants that are arranged in the population so that the downwind habitat lacks conspecifics are effectively sterile. 4) The conspecifics growing downwind from a male have a greater than random chance of being his daughters, since it is the females growing downwind from him that will be his mates and simultaneously the producers of the seeds that have moved yet further downwind.

I suspect that the above biological traits explain at least in part why *Ateleia herbert-smithii* has not spread out of its very local range as humans have destroyed the forest in the general area. Topographically, *A. herbert-smithii* occupies a ridge–mesa that runs roughly at right angles to the direction of the dry season winds (that disperse the seeds). The tree has probably not spread upwind into pastures and secondary succession simply because the seeds are wind-dispersed. Within Santa Rosa, the tree has spread downwind but has run into two kinds of inhospitable habitats: pastures that for hundreds of years have been burned repeatedly, and deciduous and/or semi-evergreen forest on land so flat that most of the disturbances are only that of tree falls. I suspect, but cannot demonstrate, that the frequency of tree falls in most kinds of pristine forest in Santa Rosa is not great enough to support a population of a dioecious wind-dispersed tree that is also wind-pollinated and does not start major reproduction until it is 20–30 years old.

The upcoming fates of the attempts to introduce it around the world as a firewood tree (Hughes & Stiles, 1984) may well be instructive on the question of why the tree has not spread away from Santa Rosa. If the tree is introduced into areas where the vegetation is relatively intact, it may well be that *Ateleia herbert-smithii* will be a very poor naturalized invader even though it is a ruderal species, in view of its obvious inability to spread in Santa Rosa. On the other hand, once it is tried on many different exotic habitats, it may well find itself in one in which it will maintain a high density irrespective of other desired uses for the site (especially if the weevil does not accompany it).

It is likely that *Apion johnschmitti* and its relatives will be found to be involved with *Ateleia* reproductive biology throughout its natural range. Whitehead (1985) found a closely related species (*Apion analomum* Kissinger) in fruits of *Ateleia gummifera* from the Bahamas, and Kissinger (1974) described this species from wild-caught beetles in Cuba, where *Ateleia gummifera* is widely distributed (Rudd, 1968). A similar but undescribed species of *Apion* was found that had developed in a flower bud of *Ateleia arsenii* (Quinceo, near Morelia, Michoacan, Mexico, 2,800 m) (Whitehead, 1985). Unfortunately, if the high percent of seed survival in the Nicaraguan population is indeed due to the anthropogenic demise of the weevil, the *Apion* that feed on *Ateleia herbert-smithii* seeds may be as endangered as are their host plants.

Apion johnschmitti plays a very large role in the biology of *Ateleia herbert-smithii* in Santa Rosa. It is easy to imagine that the tree would be more abundant within Santa Rosa if the weevil were absent. This implies that *A. herbert-smithii* would expand its range in Costa Rica if its crops of surviving seeds were much larger and it therefore had more tries at recruitment in habitats where the probability of death of a seedling is high. Ironically, the habitat destruction at the Nicaraguan population of *A. herbert-smithii* may result in an increase in recruitment for the species, not only through producing more insolated habitats for colonization, but through increasing the number of surviving seeds by making life very difficult for the weevil. *Ateleia herbert-smithii* is a plant that might do best of all as, for example, a firewood tree in tropical habitats with a strong dry season and virtually all other vegetation cleared off the site (especially vegetation that could form a fuel base for occasional fires in the dry season). It may be ideal for sites where much of the barrier to fuelwood development is collection of woody plant foliage for livestock before the wood biomass can accumulate. On the other hand, there remains the possibility that goats or other domestic herbivores might feed on *A. herbert-smithii* foliage under certain circumstances. Its weed potential in such a circumstance might well be controlled by culling female trees as they first flower. However, introduction of *Apion johnschmiti* as a means of controlling *A. herbert-smithii* does not appear to have much promise unless the vegetation is so intact that it offers sufficient refugia for the weevil between fruit crops.

Acknowledgments

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This paper is dedicated to Velva Rudd, whose serious and prompt taxonomic help encouraged me to believe that tropical plants could be seriously treated as distinct species. It is also dedicated to Arthur Bell, who inspired me to think seriously about why plants have secondary compounds in their seeds. Finally, it is dedicated to Brian Styles and Colin Hughes, who are saddled with the horrific responsibility of deciding whether this and many other tropical trees should be introduced into habitats and continents other than their native homes.

References

Arroyo, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. Royal Botanic Gardens, Kew.

Augspurger, C. K. 1986a. Morphology and dispersal potential of wind-dispersed diaspores of neotropical trees. Amer. J. Bot. 73: 353–363.

———. 1986b. Double- and single-seeded indehiscent legumes of *Platypodium elegans:* consequences for wind dispersal and seedling growth and survival. Biotropica 18: 45–50.

Bell, E. A., M. Y. Qureshi, R. J. Pryce, D. H. Janzen, P. Lemke & J. Clardy. 1980. 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine) and 2,4-methanoglutamic acid (1-amino-1,3-dicarboxycyclobutane) in seeds of *Ateleia herbert-smithii* Pittier (Leguminosae). J. Amer. Chem. Soc. 102: 1409–1412.

Dillon, M. O. 1980. *Ateleia*. Ann. Missouri Bot. Gard. 67: 551–553.

Faegri, K. & L. van der Pijl. 1979. The Principles of Pollination Ecology. Pergamon Press, Oxford.

Gladstone, D. E. 1979. Description of a seed-shadow of a wind-dispersed tropical tree. Brenesia 16: 81–86.

Halliday, J. & P. Somasegaran. 1984. The Rhizobium Germplasm Resource at NIFTAL. Catalogue of Strains. Unpublished report, University of Hawaii.

Hughes, C. E. & B. T. Styles. 1984. Exploration and seed collection of multiple-purpose dry zone trees in Central America. Int. Tree Crops J. 3: 1–31.

Janzen, D. H. 1978. Inter- and intra-crop variation in seed weight of Costa Rican *Ateleia herbert-smithii* Pitt. (Leguminosae). Brenesia 14/15: 311–323.

———. 1980. Specificity of seed-attacking beetles in a Costa Rican deciduous forest. J. Ecol. 68: 929–952.

———. 1981. Lectins and plant–herbivore interactions. Rec. Adv. Phytochem. 15: 241–258.

———. 1982a. Ecological distribution of chlorophyllous developing embryos among perennial plants in a tropical deciduous forest. Biotropica 14: 232–236.

———. 1982b. Wild plant acceptability to a captive Costa Rican Baird's tapir. Brenesia 19/20: 99–128.

———. 1988. Guanacaste National Park: tropical ecological and cultural restoration. Pp. 143–192 *in* J. Cairns, Jr. (editor), Rehabilitating Damaged Ecosystems, Volume 2. CRC Press. Boca Raton, Florida.

——— & R. Liesner. 1980. Annotated check-list of plants of lowland Guanacaste Province, Costa Rica, exclusive of grasses and non-vascular cryptograms. Brenesia 18: 15–90.

——— & P. G. Waterman. 1984. A seasonal census of phenolics, fibre and alkaloids in foliage of forest trees in Costa Rica: some factors influencing their distribution and relation to host selection by Sphingidae and Saturniidae. Biol. J. Linn. Soc. 21: 439–454.

———, C. A. Ryan, I. E. Liener & G. Pearce. 1986. Potentially defensive proteins in mature seeds of 59 species of tropical Leguminosae. J. Chem. Ecol. 12: 1469–1480.

Kissinger, D. G. 1974. Apioninae occurring in the West Indies (Curculionidae, Coleoptera). Taxonomic Publications, Riverside, California.

POLHILL, R. M. 1981. Sophoreae. Pp. 213–230 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. Royal Botanic Gardens, Kew.

_____ & P. H. RAVEN (editors). 1981. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

RUDD, V. E. 1968. A resume of *Ateleia* and *Cyathostegia* (Leguminosae). Contr. U. S. Natl. Herb. 32: 385–409.

TUCKER, S. C. 1989. Evolutionary implications of floral ontogeny in legumes. This volume.

UHL, C. & R. BUSCHBACHER. 1985. A disturbing synergism between cattle ranch burning practices and selective tree harvesting in the eastern Amazon. Biotropica 17: 265–268.

WHITEHEAD, D. R. 1985. Systematics of *Apion (Trichapion) johnschmitti*, a new species from Costa Rica (Coleoptera: Curculionoidea: Apionidae). Col. Bull. 39: 111–118.

APPENDIX I. Fruit and seed crop parameters for marked female *Ateleia herbert-smithii* in Santa Rosa National Park, 1974–1985. Each tree has the same number from year to year. Absence of data for a tree in the years after it first appears in the record means that it did not fruit, it was destroyed, or it was impossible to further census it. Absence of data for a tree between years in which it fruited means that it did not flower or fruit in the years in which it is absent from the record.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
1974							
1	25,000	252	82.54	82.54	0.00	0.00	4,365
2	120,000	232	65.09	65.09	0.00	0.00	41,897
3	100,000	258	23.64	23.64	0.00	0.00	76,357
4	75,000	283	49.12	49.12	0.00	0.00	38,163
5	150,000	294	47.73	47.73	0.00	0.00	79,081
6	50,000	160	85.00	58.12	71.43	46.00	30,625
7	150,000	256	64.84	47.84	50.29	36.00	100,195
8	90,000	288	42.01	42.01	0.00	0.00	52,188
9	50,000	304	63.49	42.23	52.65	50.00	43,421
10	50,000	278	84.89	84.89	0.00	0.00	7,554
11	70,000	1,119	77.12	52.78	38.86	46.00	48,293
12	70,000	363	39.95	39.95	0.00	0.00	42,039
13	200,000	371	78.17	78.17	0.00	0.00	43,665
14	75,000	1,205	89.63	89.63	0.00	0.00	7,780
15	50,000	257	67.70	67.70	0.00	0.00	16,148
16	100,000	245	78.37	78.37	0.00	0.00	21,633
17	20,000	260	61.15	55.79	16.67	9.00	9,692
18	200,000	314	32.80	31.50	5.36	4.00	142,675
19	100,000	305	53.12	53.12	0.00	0.00	46,885
20	50,000	276	44.57	32.80	32.94	36.00	45,652
1976							
1	14,111	611	95.75	92.86	40	3.00	1,039
1A	45,709	709	81.17	81.17	0	0.00	8,317
2	1,197	697	96.27	87.60	66	10.00	163

APPENDIX I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
3	944	444	85.36	67.56	55	12.00	387
4	1,069	569	71.35	71.23	0	0.00	308
5	1,073	573	80.98	68.24	50	19.00	397
6	14,070	570	98.42	98.42	0	0.00	173
7	23,189	689	84.04	84.04	0	0.00	3,702
8	6,137	962	98.75	98.75	0	0.00	77
9	2,946	1,038	99.04	99.31	0	0.00	28
11	270,079	859	99.07	99.07	0	0.00	2,515
12	6,650	1,250	98.56	98.56	0	0.00	96
12A	14,286	786	92.49	92.49	0	0.00	1,072
13	10,711	811	99.38	99.38	0	0.00	66
14	20,886	636	98.59	98.59	0	0.00	230
15	50,595	1,095	98.72	98.72	0	0.00	647
16	23,170	670	94.03	94.03	0	0.00	1,383
17	129,096	786	94.66	94.66	0	0.00	6,898
18	28,823	663	91.70	91.70	0	0.00	2,391
19	51,457	857	73.40	73.40	0	0.00	13,690
20	23,362	4,112	83.29	74.72	33	12.00	4,522
41	135,618	618	86.41	86.41	0	0.00	18,433
47	18,672	672	77.83	77.83	0	0.00	4,140
49	9,535	535	68.22	68.22	0	0.00	3,030
103	2,768	518	76.83	76.83	0	0.00	641
109	23,064	564	87.41	74.36	49	18.00	6,952
111	5,099	599	72.12	72.12	0	0.00	1,422
176	176,271	771	94.81	93.24	21	2.00	12,117
177	153,761	761	55.59	55.59	0	0.00	68,293
180	3,464	764	57.33	57.33	0	0.00	1,460
181	117,589	589	87.95	86.05	14	2.00	16,770
184	6,817	617	49.76	49.76	0	0.00	3,425
185	22,895	695	65.18	63.09	8	3.00	8,450
1978							
LM	67,770	7,915	77.25	77.25	not available	not available	15,420
UM	646,700	8,407	38.60	38.60			397,080
6	4,966	685	19.42	19.42			4,002
7	1,815	363	80.44	80.44			355
8	237,600	3,155	93.66	93.66			15,061
9	13,080	654	74.92	74.92			3,280
11	448,189	3,312	95.35	95.35			20,840
14	633,600	1,760	55.34	55.34			282,959
15	61,562	2,712	45.39	45.39			33,618
16	10,858	855	55.21	55.21			4,864
19	46,203	1,045	73.78	73.78			12,114

APPENDIX I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
123	10	10	0.00	0.00			10
124	16,586	887	87.71	87.71			2,038
137	13,632	568	94.72	94.72			720
138	27,602	1,302	88.40	88.40			3,201
143	18,609	1,311	96.95	96.95			568
145	10,802	867	90.31	90.31			1,047
176	283,792	969	60.68	60.68			111,584
1980							
LM	188,002	3,218	96.99	96.55	12.50	1.00	6,543
UM	426,136	3,101	90.81	90.55	2.72	0.00	40,401
1	140,910	1,281	90.71	90.29	3.20	0.00	13,750
6	43,360	542	97.42	97.42	0.00	0.00	1,120
7	1,329	443	93.45	93.03	6.45	0.00	93
8	117,682	2,029	96.80	95.99	19.51	1.00	4,756
9	9,456	788	95.69	95.32	8.11	0.00	440
11	278,190	2,529	96.13	95.89	1.92	0.00	11,440
13	3,362	747	91.43	90.96	5.88	1.00	306
14	391,416	1,938	97.63	96.98	22.03	1.00	11,916
15	28,496	1,781	90.01	89.96	0.56	0.00	2,864
16	401,866	1,509	94.63	94.44	3.57	0.00	22,370
19	10,238	1,621	79.09	79.09	0.00	0.00	2,141
109	75,590	1,665	84.57	75.16	37.58	14.00	21,383
176	183,820	1,313	86.90	85.05	12.94	2.00	28,140
177	188,416	1,472	59.44	59.44	0.00	0.00	76,418
179	10,050	1,005	90.15	90.15	0.00	0.00	990
181	7,104	592	84.97	84.11	4.21	1.00	1,140
184	26	26	0.00	0.00	0.00	0.00	0
200	100,700	2,090	97.70	97.56	5.88	0.00	2,457
201	20,608	681	96.62	96.62	0.00	0.00	696
202	53,952	1,124	96.09	94.66	19.67	2.00	2,908
203	11	11	0.00	0.00	0.00	0.00	0
207	49,020	817	87.27	87.27	0.00	13.00	1
208	125,184	1,956	94.02	94.02	0.00	0.00	7,489
1982							
LM	354,552	11,130	98.74	98.18	8.87	0.00	6,467
UM	1,204,000	11,113	97.89	97.83	2.49	0.00	26,110
6	83,250	2,775	99.06	99.06	0.00	0.00	780
7	347,131	4,342	96.04	95.88	0.56	0.00	14,311
8	40,964	3,723	99.44	99.41	4.55	0.00	242
9	374,896	3,392	97.94	97.91	0.00	0.00	7,847
11	838,590	35,015	97.54	97.44	3.34	0.00	21,531

APPENDIX 1. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
12	29,598	4,656	97.23	97.05	2.90	0.00	877
13	64,239	6,375	97.91	97.76	6.99	0.00	1,443
14	217,984	6,212	97.46	97.27	7.65	0.00	5,965
15	89,162	4,501	98.56	98.53	0.00	0.00	1,307
16	288,805	4,355	97.08	96.66	11.64	0.00	9,682
19	255,210	4,593	98.15	98.15	0.00	0.00	4,721
20	616	616	89.61	88.47	6.76	4.00	74
123	33,698	1,489	97.58	91.96	70.87	6.00	2,874
124	108,460	1,972	98.23	95.04	61.39	3.00	5,550
126	45,795	2,130	97.09	96.91	6.06	0.00	1,419
127	111,022	3,747	98.03	95.25	57.38	3.00	5,422
128	4,432	956	95.50	95.20	6.52	0.00	213
129	4,839	1,281	97.11	96.96	5.13	0.00	147
130	1,944,000	4,860	98.79	98.62	11.94	0.00	26,800
137	79,241	2,308	99.22	99.14	10.00	0.00	687
138	45,486	2,909	97.22	97.18	2.44	0.00	1,282
145	35,785	2,302	99.22	99.18	5.26	0.00	295
147	66,814	2,197	97.36	95.84	16.00	2.00	2,281
176	376,800	4,422	97.22	96.61	14.57	1.00	12,867
177	468,545	3,383	98.94	98.91	2.70	0.00	5,124
179	30,712	1,118	98.21	98.21	0.00	0.00	549
180	18,282	2,210	92.53	92.41	1.19	0.00	1,390
181	153,964	39,044	94.39	91.99	28.71	3.00	12,694
184	771	771	76.27	75.77	0.54	1.00	185
185	3,365	3,365	96.46	95.84	13.48	1.00	141
200	120,608	2,774	98.77	98.77	0.00	0.00	1,478
201	238,073	7,132	97.29	97.13	5.37	0.00	6,843
202	314,104	3,976	95.57	94.79	13.88	16.00	16,511
251	30,992	2,945	97.96	97.96	0.00	0.00	631
253	103,785	1,785	97.09	95.23	39.08	2.00	5,058
255	45,408	2,838	94.89	93.66	14.21	2.00	2,928
258	1,421	758	97.49	97.49	0.00	0.00	36
261	75,516	2,436	94.17	93.75	6.54	0.00	4,743
271	6,409	1,989	94.92	93.10	25.00	2.00	451
272	1,721	1,052	93.73	93.46	2.90	0.00	113
273	120,516	5,478	92.95	91.23	19.39	2.00	10,780
274	385,125	5,135	96.92	96.83	3.03	0.00	12,375
275	49,245	3,283	98.45	98.30	8.93	0.00	840
276	21,014	1,501	96.74	96.67	2.00	0.00	700
277	94,826	2,734	98.54	93.97	76.30	5.00	6,000
278	10,246	1,636	95.29	79.54	78.71	20.00	2,530
279	112,087	5,579	96.70	96.67	1.08	0.00	3,737
280	267,599	3,769	97.75	90.77	75.73	8.00	26,625
281	63,842	2,895	96.72	93.59	45.83	3.00	4,234
282	369,840	4,129	97.51	97.20	10.35	0.00	10,390

APPENDIX I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
283	61,327	1,649	94.66	92.00	33.09	3.00	5,058
284	45,128	1,223	93.46	93.38	1.24	0.00	2,989
285	1,102,152	3,909	96.65	95.76	19.64	1.00	47,368
286	330,852	2,830	92.69	91.81	9.83	1.00	27,357
287	124	124	79.03	79.03	0.00	0.00	26
288	69,285	1,455	95.40	93.36	30.30	2.00	4,714
289	42,714	3,051	94.43	93.98	7.57	1.00	2,590
290	69,326	1,384	98.41	97.15	45.00	1.00	2,064
291	36,897	1,757	94.42	83.96	66.04	14.00	6,741
292	293,121	2,969	98.05	97.85	9.09	0.00	6,516
293	23,697	2,633	98.03	97.99	1.89	0.00	477
294	642,488	5,959	97.57	94.93	51.77	3.00	33,531
295	41,198	2,612	98.28	96.52	47.37	4.00	1,498
296	34,960	4,370	25.17	24.96	0.15	1.00	26,512
296 repl.	11,157	11,157	31.16	30.96	0.25	1.00	7,760
297	8,883	1,269	88.57	88.50	0.00	0.00	1,022
298	49,078	2,438	96.39	96.35	1.12	0.00	1,792
299	20,192	5,235	95.91	95.84	0.92	0.00	841
300	66,302	10,664	95.29	95.11	2.87	0.00	3,252
301	12,330	4,110	99.39	99.39	0.00	0.00	75
302	367,392	8,544	99.16	99.15	0.00	0.00	3,139
303	484,763	7,164	98.91	98.83	0.00	0.00	5,684
304	27,293	3,560	99.47	99.47	0.00	0.00	146
305	39,051	4,409	99.07	99.07	0.00	0.00	363
306	167,901	5,916	97.90	97.69	8.03	0.00	3,888
307	314,221	4,096	98.68	98.40	12.12	0.00	5,063
308	56,559	3,327	97.54	97.01	16.00	1.00	1,700
309	111,052	1,214	98.93	98.93	0.00	0.00	1,189
310	2,958	1,450	89.24	84.16	28.57	7.00	500
311	155,545	5260	97.15	97.06	2.58	0.00	4,584
312	236,691	4,434	98.17	97.87	11.58	0.00	5,071
313	47,800	8,365	98.84	98.76	4.81	0.00	594
314	51,554	2,999	96.40	95.51	18.98	2.00	2,355
315	374,595	7,345	98.29	98.22	2.29	0.00	6,681
316	43,927	3,404	97.27	97.21	2.11	0.00	1,226
317	627	627	96.81	96.82	0.00	0.00	20
318	309,886	8,049	97.24	97.16	1.31	0.00	8,816
319	440,510	6,082	98.78	98.70	6.25	0.00	5,794
320	509,929	6,099	98.16	98.04	2.50	0.00	10,033
321	884	884	92.53	92.43	2.99	0.00	67
322	821	821	88.55	88.55	0.00	0.00	94
323	9,557	9,557	96.51	96.46	0.59	0.00	339
324	58,242	3,426	98.57	98.48	1.92	0.00	884
325	144,904	3,304	98.79	98.02	30.30	1.00	2,895

Appendix I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
326	175,497	2,887	99.17	98.99	13.79	0.00	1,769
327	7,074	2,358	99.32	99.32	0.00	0.00	48
328	60,418	1,841	98.75	98.64	8.00	0.00	820
329	348,227	19,874	92.58	92.33	2.55	0.00	26,790
330	295,079	3,053	99.44	99.44	0.00	0.00	1,643
1984							
LM	3,972	538	95.72	95.74	0.00	0.400	170
UM	5,642	830	98.44	98.44	0.00	0.200	88
1	34,053	1,570	98.22	97.97	12.50	0.300	694
6	12,738	1,019	97.55	97.55	0.00	0.000	313
8	6,670	2,001	98.25	98.15	5.41	0.100	123
14	9,310	1,330	94.89	93.97	16.05	1.000	567
15	2,413	724	95.72	95.45	6.06	0.300	110
16	165,205	2,423	98.80	98.60	14.71	0.200	2,318
124	181,769	5,857	95.10	93.63	22.16	1.600	1,762
130	14,400	1,008	99.40	99.40	0.00	0.000	86
130 repeat	9,286	650	58.31	58.13	0.73	0.300	3,900
137	25,417	1,112	97.12	97.10	0.00	0.600	731
143	9,786	1,896	95.99	95.94	1.30	0.100	397
145	23,965	2,716	97.94	97.79	3.33	0.200	529
147	74,032	2,311	95.33	94.48	14.73	1.100	4,132
176	139	139	41.73	41.73	0.00	0.000	81
200	760	380	98.95	98.95	0.00	0.000	8
272	38	38	71.05	71.05	0.00	0.000	11
293	129	129	91.47	91.47	0.00	0.000	11
294	444,814	17,189	93.75	92.34	19.07	1.600	34,443
298	1,061	467	97.00	96.18	22.22	0.800	41
302	3,195	703	98.58	98.58	0.00	0.000	45
303	266	266	79.70	79.70	0.00	0.000	54
306	4,915	1,413	97.66	97.60	2.94	0.100	118
309	16,359	1,854	97.20	97.09	1.85	0.100	476
311	9,300	992	99.60	99.60	0.00	0.000	38
312	1,415	566	92.76	92.43	2.33	0.400	108
313	6,065	4,380	98.33	98.33	1.35	0.000	102
314	3,212	835	96.05	94.61	21.74	2.300	177
318	3,765	1,705	95.13	95.13	0.00	0.000	183
319	3,987	1,196	98.33	98.33	0.00	0.000	67
320	13,148	1,157	97.93	97.84	4.00	0.100	284
325	21,071	1,475	84.81	84.81	0.00	0.000	3,200
328	11,616	968	95.45	95.36	2.22	0.100	540
400	161,108	10,155	95.01	94.92	1.36	0.100	8,186

APPENDIX I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
401	51,976	5,291	90.95	90.84	0.82	0.100	4,764
402	47,572	4,061	77.35	68.02	29.44	17,400	17,864
1985							
LM	922,208	5,728	98.20	98.06	8.04	1.00	18,032
UM	565,200	8,851	97.30	95.39	16.83	2.00	26,565
6	9,486	1,165	98.37	98.03	13.04	0.00	187
7	2,187	767	94.92	94.81	2.50	1.00	114
8	2,239	684	98.83	98.83	0.00	0.00	26
11	293,797	48,056	97.77	97.59	6.12	0.00	7,092
12	6,192	1,032	97.77	97.50	7.69	1.00	156
13	17,622	1,958	98.72	98.38	21.88	1.00	288
14	36,835	2,085	97.51	96.85	22.39	2.00	1,184
15	12,047	3,681	98.59	98.43	10.34	0.00	190
16	13,475	1,225	99.35	99.35	0.00	0.00	88
19	15,632	1,839	97.66	97.40	10.42	0.00	408
20	30,243	2,412	98.51	92.94	79.70	18.00	2,533
123	17,919	594	97.64	96.20	30.43	2.00	694
128	76,979	4,586	97.47	93.76	53.42	7.00	5,153
129	36,493	2,546	98.11	98.00	1.96	0.00	731
130	44,678	1,992	97.04	96.43	11.11	1.00	1,615
137	1,863	138	98.55	97.84	33.33	1.00	41
143	4,780	493	98.38	98.38	0.00	0.00	78
147	8,253	917	97.27	96.15	25.00	2.00	324
176	20,108	1,828	97.76	97.25	15.69	1.00	561
177	5,756	999	98.00	98.00	0.00	0.00	115
180	1,439	365	99.18	99.18	0.00	0.00	12
185	3,056	904	92.92	92.65	1.49	1.00	227
200	2,180	1,387	98.92	98.50	28.57	1.00	33
202	40,013	2,200	98.27	96.63	46.75	4.00	1,400
251	2,831	1,514	98.41	98.22	7.41	0.00	50
253	29,499	1,189	97.65	97.02	38.89	3.00	893
255	21,009	2,251	98.62	96.91	52.78	3.00	672
258	22,222	2,292	97.99	97.91	2.08	0.00	465
261	7,836	1,413	98.87	98.39	30.43	1.00	128
263	6,608	1,279	98.05	97.74	13.79	1.00	150
267	84,727	4,660	97.83	96.38	38.15	3.00	3,145
269	13,844	558	98.21	97.35	26.67	1.00	372
270	78,529	6,813	98.55	96.98	51.66	3.00	2,432
271	2,077	1,111	98.83	97.87	45.83	1.00	45
273	62,509	3,677	98.40	96.58	54.26	2.00	2,193
274	11,806	2,654	98.38	97.98	18.52	1.00	240
275	8,780	1,211	97.61	97.05	19.44	1.00	261
281	5,000	1,124	98.58	97.72	38.46	1.00	116

APPENDIX I. Continued.

Tree number	Estimated fruit crop size	Number of fruit sampled	Percent of fruit attacked	Percent of seeds killed	Percent of seeds surviving because in 2-seeded fruit	Percent of crop 2-seeded	Number of seeds surviving
285	57,577	2,062	98.01	96.96	34.38	2.00	1,787
286	12,936	616	98.70	98.71	0.00	0.00	168
295	22,846	2,264	97.66	95.00	48.91	21.00	1,382
296	1,685	822	95.86	93.06	41.67	4.00	123
300	17,908	1,847	97.89	96.82	35.00	2.00	582
301	762	501	97.41	97.41	0.00	0.00	20
302	6,412	1,241	98.07	97.75	7.14	0.00	145
303	19,318	1,862	98.60	98.50	7.14	0.00	291
305	7,568	688	98.26	98.84	0.00	1.00	88
306	28,728	3,078	97.17	96.77	13.00	0.00	933
307	99,424	3,824	98.17	93.73	71.43	11.00	6,916
308	4,412	938	98.40	95.17	66.67	6.00	226
310	1,004	1,004	96.81	89.97	70.94	16.00	117
312	11,331	1,259	98.57	98.10	25.00	0.00	216
313	7,056	2,352	98.81	98.77	0.00	0.00	87
314	7,388	1,019	98.43	97.86	27.27	1.00	160
315	7,185	712	98.03	97.50	16.67	1.00	182
318	4,145	1,306	97.32	97.18	5.41	0.00	117
319	9,477	620	99.03	97.95	38.46	2.00	199
320	30,173	2,235	99.15	99.06	9.52	0.00	284

APPENDIX II. Diameter (dbh) increment per year by healthy adult male *Ateleia herbert-smithii* in Santa Rosa National Park, January 1983 to January 1985.

Tree number	Initial diameter (cm)	Increment/year (cm)
250	62.99	0.64
252	57.91	0.30
254	46.23	0.58
256	49.28	0.41
257	40.89	0.54
259	30.99	0.69
260	51.31	0.07
262	50.55	0.10
265	50.29	0.29

APPENDIX III. Diameter (dbh) increment per year by adult healthy female *Ateleia herbert-smithii* in Santa Rosa National Park, January 1983 to January 1985.

Tree number	Initial diameter (cm)	Increment/year (cm)
LM	35.56	0.71
6	39.12	0.59
7	50.80	0.21
8	20.57	0.39
9	60.71	0.27
13	22.35	1.55
15	26.67	1.08
16	70.36	0.38
123	46.48	1.46
124	50.04	0.07
130	62.23	0.81
176	42.93	0.01
177	35.31	0.14
179	20.83	0.83
180	22.61	0.46
184	12.70	0.37
185	10.92	0.05
200	27.94	0.49
201	24.38	0.51
251	24.89	0.19
253	51.82	0.56
255	29.46	0.61
258	36.32	0.42
261	18.29	0.26
263	17.27	0.89
264	28.45	0.41
267	33.27	0.18
268	11.18	0.23
269	46.48	0.13
270	36.58	2.90
271	21.59	1.79
273	18.54	0.21
274	34.54	0.22
275	26.67	0.45
276	50.80	0.46
277	32.00	0.72
278	17.27	0.26
280	52.07	0.51
281	32.26	0.49
282	31.50	0.48
283	38.86	0.46
284	39.12	0.23
285	56.64	0.14
287	14.73	0.49
288	25.40	0.28
289	23.88	0.25
290	61.47	1.30
291	42.67	0.00
293	21.59	0.47
294	71.37	0.36

APPENDIX III. Continued.

Tree number	Initial diameter (cm)	Increment/year (cm)
295	17.53	0.23
296	21.34	1.21
298	29.21	1.25
299	18.03	0.69
300	22.35	1.27
302	33.27	0.39
303	38.35	0.09
304	23.11	0.49
305	23.62	0.07
306	43.94	2.30
307	48.77	0.46
308	28.96	1.16
309	58.93	0.05
310	18.29	1.30
311	35.81	0.60
312	38.10	0.12
313	16.51	0.09
314	29.97	0.21
315	38.61	0.27
316	27.18	0.35
317	10.67	0.42
319	33.27	0.24
320	46.74	0.12
321	7.11	0.54
322	8.38	0.71
323	6.10	0.30
325	35.81	0.92
328	35.56	0.18

APPENDIX IV. Diameter (dbh) increment per year by healthy female *Ateleia herbert-smithii* in Santa Rosa National Park, January 1977 to January 1985.

Tree number	Initial diameter (cm)	Increment/year (cm)
11	55.12	0.32
16	66.04	0.64
124	48.01	0.27
128	37.85	1.47
129	27.69	1.31
130	59.69	0.52
138	87.12	0.39
143	49.02	0.22
145	38.86	0.10
147	33.27	0.35
176	37.34	0.70
177	32.77	0.35
179	15.75	0.84
180	20.57	0.37
184	11.94	0.19
185	10.41	0.08

APPENDIX V. Fruit crop parameters for the 459 terminal infructescences on one major branch of *Ateleia herbert-smithii* number 181 in 1982. All seed death was caused by *Apion johnschmitti*. Blanks are zeros. Note: branch numbers were assigned as the branches were taken from a large but nonrandomized branch pool and should be randomized before subsampling.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
1	99	10	2		
2	25	1	2	1	
3	81	1	12	1	
4	102	5	4	5	
5	115	6	7		
6	87	8	1	1	
7	57	4	4		
8	54	1	0		
9	91	5	1	1	
10	170	6	1		
11	122	5	6	1	
12	124	7	5	1	1
13	124	4	1		
14	139	9	16	1	4
15	81	3	0		
16	54	4	3	1	2
17	50	5	1		
18	57	1	2	1	2
19	39	5	1		2
20	161	12	2		1
21	158	10	6	1	
22	37	5	1		
23	160	9	5		1
24	202	9	5		1
25	133	14	10		4
26	20	1	0		
27	46	5	5	1	1
28	398	12	6		
29	107	4	3		1
30	71	9	3	1	1
31	73	7	1		1
32	57	1	1		1
33	46	2	0		
34	34	2	0		
35	168	11	10		2
36	56	0	0		
37	66	2	0	1	
38	158	8	7		
39	20	4	1		
40	62	4	1		
41	19	1	0		
42	152	8	4		
43	136	11	5	1	2
44	49	0	2		
45	125	4	4		
46	69	4	2		

Appendix V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
47	65	5	0		
48	29	0	1		
49	18	3	0		
50	33	0	0		
51	67	5	0		
52	98	4	2		
53	106	4	2		
54	32	1	2	1	
55	24	0	0		
56	112	3	0		
57	82	2	3		
58	36	0	0		
59	23	2	0		
60	209	16	14	1	1
61	67	1	2		
62	92	6	1		2
63	84	4	1		1
64	19	2	0		
65	14	0	0		
66	97	2	2		
67	94	1	2		1
68	190	11	8		3
69	103	3	0		1
70	52	1	0		
71	53	1	0		
72	56	6	0	1	
73	22	0	2	1	
74	14	3	0		
75	103	6	1		
76	86	2	0		
77	127	11	7	1	2
78	81	6	4	1	
79	89	4	1		
80	109	7	7	1	
81	123	11	6		
82	16	0	0		
83	63	2	0		
84	27	2	0		
85	18	2	0		
86	70	2	2		
87	47	1	0		
88	21	0	0		1
89	136	11	8		
90	26	0	0		
91	37	1	1		
92	120	14	5		
93	24	2	1	1	1
94	18	2	0		
95	111	6	0		
96	65	0	0		1

APPENDIX V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
97	9	1	0		
98	30	2	0		
99	90	6	3		
100	43	1	0		
101	24	3	0		
102	58	2	4		4
103	90	2	1	2	
104	230	12	2		
105	77	3	0		
106	25	5	0		
107	63	1	0		
108	66	4	2	1	
109	152	3	2		
110	82	2	0		
111	33	2	2		
112	80	5	1		
113	41	0	0		
114	48	2	1		
115	78	3	4		
116	86	2	2		
117	55	3	3	1	
118	34	1	0		
119	138	8	8	2	
120	88	1	0		
121	13	0	0		
122	8	1	0		
123	110	5	6	2	
124	58	3	0		
125	174	8	9		
126	82	3	1		
127	110	5	3		
128	172	7	5	1	1
129	103	7	4		1
130	67	10	11	2	
131	91	3	3		
132	37	3	4		
133	75	3	1		
134	54	1	1		
135	121	6	10	1	
136	112	4	1		1
137	22	1	0		
138	55	2	1		
139	269	14	4		2
140	65	5	6		
141	65	3	1		
142	69	1	5		1
143	72	2	2	1	
144	114	6	5		
145	59	4	1		
146	221	16	5	2	

Appendix V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
147	233	10	7		
148	156	6	8	1	
149	194	22	6	1	
150	55	6	4		
151	114	3	0		
152	41	0	1		
153	36	3	2		
154	107	3	1	1	
155	158	13	6		1
156	44	3	0		
157	118	9	3		
158	51	2	1	1	
159	69	3	4		
160	24	1	2		
161	71	1	2	1	1
162	54	1	1		
163	84	7	3		1
164	14	2	0		
165	28	6	1		
166	36	2	2		
167	17	0	0		
168	13	8	2		
169	23	4	0		
170	84	5	1		
171	94	1	1		1
172	177	9	14	1	
173	149	15	2	2	1
174	121	5	1		
175	22	0	0		
176	63	4	2	2	
177	117	2	1		
178	84	3	0		
179	204	11	1		
180	154	9	8		1
181	152	8	1		
182	136	2	0		
183	133	4	2		
184	100	8	2		
185	87	3	1		
186	60	3	2		
187	45	9	1		
188	109	5	0	1	
189	160	3	8		
190	119	5	6		1
191	163	4	0	1	1
192	69	2	0	1	
193	155	2	4		
194	66	5	2		1
195	56	3	1		
196	238	9	10		2

Appendix V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
197	43	3	1	1	
198	49	1	1		
199	102	2	0		
200	146	7	7		
201	125	4	6		
202	143	8	4		
203	224	10	5		3
204	121	5	1		
205	20	0	0		
206	501	22	14	3	3
207	13	0	0		
208	32	1	0		
209	354	22	11	1	
210	26	1	1		
211	27	0	0		
212	63	0	0		
213	22	2	1		
214	44	1	0		
215	56	1	0	1	
216	157	8	0		1
217	113	4	7		
218	106	4	0	1	1
219	84	10	5		1
220	63	2	0		
221	92	12	1	1	1
222	95	5	1		1
223	98	0	2		
224	29	2	1		
225	143	7	3	2	1
226	77	7	0		
227	13	3	0		
228	113	1	5		
229	5	0	0		
230	324	12	8	1	1
231	41	0	1		
232	36	1	0		
233	30	4	1		
234	594	35	12	2	
235	174	3	5		
236	45	0	0		
237	19	0	1		
238	53	1	4		
239	26	2	1		
240	13	3	1		
241	50	0	0		
242	54	4	0		1
243	8	1	1		
244	61	5	0		
245	136	18	5		1
246	112	3	0		

APPENDIX V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
247	49	3	1		
248	53	3	0		
249	36	1	0		
250	16	0	0		
251	77	6	1		
252	64	6	0		
253	48	0	0		
254	37	3	2		
255	65	1	1		
256	73	5	5		2
257	54	2	2		
258	18	1	0		
259	26	1	1		
260	98	2	1		
261	206	19	4		
262	83	5	2	1	
263	33	4	0		
264	22	2	0		
265	64	1	0		
266	316	22	3		
267	93	15	1		
268	30	0	0		
269	21	0	0		
270	62	3	0		
271	28	2	0		
272	92	2	0		
273	273	26	4		
274	17	1	0	1	
275	58	3	0		
276	264	12	1	1	1
277	25	7	0		
278	38	5	0		
279	26	4	2		
280	206	15	4		
281	37	0	0		
282	40	4	1	1	
283	170	8	1	2	
284	94	1	2		
285	82	8	0		
286	62	8	4		
287	190	12	3		
288	82	4	1		
289	32	2	0		
290	28	1	0		
291	46	2	2		
292	84	6	1		
293	26	1	0		
294	130	11	3	1	
295	13	1	0		
296	56	1	0		

Appendix V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
297	15	4	1		
298	140	8	3	1	
299	76	1	4		1
300	87	5	1		1
301	36	1	0		
302	16	2	0		
303	37	6	0		
304	54	1	1		
305	38	6	0		
306	23	1	0		
307	19	1	1		
308	62	5	3		
309	55	3	0		
310	28	1	0	1	
311	23	1	0		
312	34	26	1	1	
313	17	2	0		
314	58	11	2		
315	37	6	0	1	
316	21	1	0		
317	17	2	0		
318	10	2	0		
319	16	0	0		
320	13	2	0		
321	12	2	0		
322	53	2	1		
323	24	1	0		
324	89	29	2		
325	32	3	0		
326	27	14	3		
327	18	1	0		
328	100	7	3		
329	43	3	0	1	
330	28	5	2		
331	26	0	0	1	
332	13	11	0		
333	38	2	1		
334	14	3	0		
335	30	12	0	1	
336	14	0	0		
337	107	8	4	1	1
338	8	0	0		
339	44	1	1		
340	36	2	0		
341	34	1	0		1
342	41	4	1		
343	20	2	0		
344	12	4	0		
345	9	0	0		
346	17	5	0		

Appendix V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
347	88	2	2		
348	56	3	2		1
349	26	0	0		
350	100	4	1		1
351	69	4	3		
352	58	1	0		
353	41	3	0		
354	116	7	2		
355	56	1	0		
356	166	10	1		
357	126	3	1		
358	37	1	2		
359	35	3	2	1	
360	119	3	3		
361	131	5	2		
362	37	2	0		
363	48	3	3		
364	29	1	0		
365	62	2	0		
366	29	3	0		
367	57	3	2		
368	18	0	0		
369	55	4	0		
370	117	8	3		3
371	50	0	2		
372	136	7	3		
373	42	1	1		
374	64	2	4		
375	110	4	0		
376	98	10	6		
377	66	2	2		1
378	106	9	1		
379	71	4	1		1
380	73	9	8		
381	60	7	0		
382	75	9	6	1	
383	47	6	1		
384	33	2	0		
385	147	6	3		
386	62	0	0		1
387	136	6	5		1
388	212	12	4	1	
389	44	3	1	2	1
390	108	5	1		
391	54	5	2		
392	32	2	0		
393	72	2	1		
394	74	4	1		
395	135	3	1		2
396	130	9	8		1

APPENDIX V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
397	60	7	0	1	
398	51	1	0	1	
399	126	9	3		
400	213	20	3	5	
401	77	6	1		
402	61	5	0		
403	148	12	4		1
404	47	4	1	1	
405	106	8	1		
406	23	1	0		
407	58	2	4		
408	209	11	7		1
409	137	1	2		
410	90	3	5		
411	105	6	4	1	
412	18	1	0		
413	65	4	1		
414	41	2	1		
415	187	14	11	2	1
416	101	4	0		1
417	99	6	1		
418	86	7	3	1	
419	89	2	1		
420	68	7	3		2
421	132	9	8	1	
422	172	10	0	1	
423	58	11	4	1	
424	76	1	0		
425	37	2	9	1	
426	97	13	12		
427	28	1	0		
428	62	4	4		
429	65	8	5		
430	39	2	0		
431	57	2	0		
432	98	10	2		
433	86	4	0		1
434	62	1	0		
435	16	0	2		
436	52	0	0		
437	44	3	0		
438	98	1	2		
439	38	1	1		
440	22	1	1		
441	40	1	2		
442	141	4	5		1
443	34	2	0	1	
444	32	2	3		
445	37	2	2		
446	88	5	1		

APPENDIX V. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
447	32	0	0		
448	29	1	0		
449	32	4	0		
450	40	4	1		1
451	113	5	2		
452	91	4	2	2	
453	93	11	2		
454	156	11	5		
455	183	9	2		
456	129	4	2		4
457	71	2	1		
458	86	1	2		1
459	77	4	2		

APPENDIX VI. Fruit crop parameters for the 500 terminal infructescences on a majority and complete subset of one major branch of *Ateleia herbert-smithii* number 11 in 1982. All seed death was by *Apion johnschmitti*. Blanks are zeros. Note: branch numbers were assigned as branches were taken from a large but nonrandomized branch pool and should be randomized before subsampling.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
1	193	3			not available
2	268	9			
3	441	16			
4	210	7			
5	299	17			
6	215	4			
7	662	9	3		
8	147	3			
9	742	29			
10	229	9			
11	525	11			
12	274	4	1		
13	201	14			
14	568	18	1	1	
15	820	26	3		
16	424	19			
17	32	1			
18	352	11			
19	202	6		1	
20	390	10	2	1	
21	150	2	1		
22	274	9	2		
23	224	4			
24	224	6			
25	162	2			

Appendix VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
26	341	7			
27	89	2			
28	113	3			
29	315	7			
30	494	11			
31	238	10			
32	158	8			
33	142	4			
34	508	23	2		
35	155	2			
36	211	5		1	
37	608	19			
38	209	4			
39	253	5			
40	194	8			
41	141	3			
42	142	6	1		
43	467	25			
44	133	3			
45	215	4			
46	196	9			
47	215	4			
48	312	10			
49	269	7			
50	360	8	2		
51	564	15	1		
52	573	6			
53	500	24			
54	200	16			
55	214	7			
56	272	9			
57	486	11	3		
58	413	9			
59	367	13	1	1	
60	202	9			
61	384	14			
62	222	6			
63	241	5			
64	275	8			
65	102	1			
66	354	5			
67	257	8			
68	38	1			
69	271	8			
70	404	7	2		
71	215	7			
72	314	19			
73	467	21	1		
74	297	5			
75	300	6			

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
76	479	19	1		
77	396	8			
78	210	3			
79	243	11			
80	112	4			
81	86	3			
82	307	11			
83	197	6	2		
84	320	5			
85	152	2	1		
86	224	5	1	1	
87	319	12			
88	248	13		1	
89	99	6			
90	333	17	3		
91	332	15			
92	44	0			
93	179	3			
94	268	7			
95	132	2	1		
96	150	1			
97	73	2			
98	183	7		1	
99	153	12			
100	140	0		1	
101	218	9			
102	72	3			
103	70	1			
104	136	4			
105	151	6			
106	173	5			
107	60	1			
108	59	7	1		
109	130	6			
110	43	4	3		
111	116	2	1		
112	92	2			
113	120	4			
114	163	7			
115	202	8			
116	180	3	2		
117	175	9			
118	182	5	1		
119	194	3		1	
120	188	5			
121	202	15			
122	238	10			
123	145	3			
124	197	9			
125	261	9		1	

Appendix VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
126	271	6			
127	163	0			
128	195	7			
129	169	12			
130	121	4			
131	215	4			
132	300	8			
133	255	10			
134	275	7			
135	296	6			
136	254	8			
137	264	5			
138	223	14	1		
139	235	7			
140	193	7			
141	261	14			
142	241	11			
143	225	2			
144	308	9			
145	281	18	1		
146	248	12	2		
147	327	16			
148	392	6	1		
149	422	20	1	1	
150	384	15			
151	312	14			
152	458	14	1		
153	356	11			
154	389	10	1		
155	437	20			
156	543	14	1		
157	393	11			
158	281	7	1		
159	277	13	1		
160	336	12			
161	363	13	1		
162	390	10			
163	555	22			
164	464	14	1		
165	401	19			
166	493	9	2		
167	535	26			
168	651	27	2		
169	496	28			
170	403	11	2		
171	752	26	2	1	
172	583	27	4		
173	720	13	1		
174	759	28	7		
175	593	10	2	1	

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
176	719	27	1	1	
177	1,052	26		1	
178	1,386	42	2	2	
179	264	12			
180	366	10			
181	336	10			
182	667	25	1		
183	314	10			
184	363	13	1	1	
185	287	16		1	
186	433	11	1		
187	366	11			
188	234	3			
189	276	7			
190	270	13	2		
191	337	10	1		
192	339	16	1		
193	349	10			
194	352	33	1		
195	414	13	1		
196	309	17			
197	490	24			
198	790	9		1	
199	241	13			
200	271	11			
201	351	29			
202	500	17	1		
203	338	11			
204	532	12	1	2	
205	144	6			
206	276	11	1		
207	965	38	1		
208	699	16			
209	107	4			
210	271	5	1		
211	243	1			
212	283	3			
213	301	9			
214	220	3	1		
215	390	10			
216	180	3			
217	748	21	2	1	
218	83	4			
219	345	8			
220	714	9			
221	274	7			
222	452	16	1		
223	73	1			
224	77	1			
225	211	4			

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
226	179	5			
227	1,044	30	1	1	
228	353	17	1		
229	252	4		2	
230	155	8			
231	52	0			
232	311	12			
233	103	3			
234	89	5			
235	472	11			
236	405	11		1	
237	132	5			
238	96	4			
239	181	8			
240	144	2			
241	148	6			
242	60	3			
243	424	13		1	
244	639	31	1		
245	259	9			
246	127	0	1		
247	44	1			
248	922	22	2		
249	338	10			
250	185	6	1		
251	450	16	1		
252	545	21	2		
253	219	12			
254	460	23	1		
255	255	12	1		
256	775	29	3		
257	633	21			
258	293	15			
259	229	8	1		
260	1,083	29	4		
261	276	5			
262	228	4			
263	378	16	2	1	
264	364	10	1		
265	391	14	2		
266	232	9			
267	259	16			
268	219	6			
269	325	10			
270	201	9			
271	403	31	1		
272	335	8			
273	360	13			
274	441	14			
275	267	10	1		

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
276	690	18		1	
277	583	17			
278	422	12	1		
279	517	6			
280	305	4			
281	424	4		1	
282	417	15			
283	138	1			
284	149	6			
285	508	14			
286	539	13			
287	361	10			
288	177	11			
289	266	9	1		
290	206	3			
291	266	1	1		
292	626	20			
293	120	1			
294	462	16	1		
295	542	12			
296	239	5			
297	239	7			
298	343	10		1	
299	220	5			
300	774	30	1	3	
301	853	24			
302	441	14			
303	364	10			
304	307	9			
305	549	11			
306	256	6			
307	711	17	2	1	
308	275	6	2	2	
309	176	8			
310	148	0			
311	408	11			
312	692	26			
313	528	15	1		
314	668	23	1		
315	249	2			
316	413	6			
317	458	24	1	2	
318	457	21	1	1	
319	556	17	2	2	
320	676	28			
321	346	10		1	
322	317	10	1		
323	132	4	1		
324	641	25	3	1	
325	187	10		1	

Appendix VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
326	502	13	2	1	
327	409	11		1	
328	596	32			
329	215	4	1		
330	307	13			
331	307	8		2	
332	695	16		2	
333	88	4			
334	383	10	3		
335	394	12	2		
336	436	13			
337	413	10		1	
338	256	10	1		
339	303	8	2		
340	44	0			
341	193	17			
342	415	6	2		
343	79	2			
344	739	8			
345	693	25			
346	81	1			
347	580	19			
348	577	17	2		
349	307	4			
350	114	3			
351	536	26			
352	12	0			
353	285	11			
354	151	7			
355	220	7			
356	405	20			
357	416	12	2		
358	817	21			
359	374	11			
360	49	6			
361	751	27	2		
362	219	12			
363	688	11			
364	405	11			
365	494	16			
366	630	12	1		
367	707	9		1	
368	416	17			
369	666	31	1		
370	400	19			
371	353	4			
372	183	4			
373	56	5			
374	194	3			
375	98	5	1		

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
376	586	17	1	2	
377	519	19			
378	175	1			
379	489	11			
380	210	2			
381	150	5			
382	150	6			
383	250	12			
384	650	11	2		
385	319	8	1		
386	123	8			
387	257	12			
388	190	7			
389	223	2			
390	341	12			
391	120	0			
392	420	4			
393	309	7	1		
394	88	1			
395	180	5			
396	306	10			
397	318	9			
398	234	11			
399	459	6			
400	130	2			
401	335	7			
402	188	2	2		
403	648	10			
404	150	3			
405	452	14			
406	201	3			
407	243	1			
408	280	9			
409	388	13	1	1	
410	52	2			
411	334	4			
412	194	5			
413	432	3			
414	158	3			
415	489	7	1	1	
416	327	1	1		
417	97	0	1		
418	46	0		1	
419	451	7			
420	197	10			
421	284	2			
422	296	8	4		
423	222	4	1		
424	230	3			
425	104	2	2		

Appendix VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
426	205	3			
427	35	1			
428	303	6			
429	321	7	1	1	
430	174	1			
431	301	10		1	
432	530	9	1		
433	439	6	1		
434	500	6			
435	438	5			
436	303	5			
437	580	8	1		
438	246	7			
439	521	10	3		
440	168	9			
441	410	7	1		
442	292	8			
443	126	1	1		
444	362	5			
445	225	5	1		
446	433	11	1		
447	395	4			
448	621	21			
449	634	24		2	
450	354	4			
451	356	13			
452	425	10			
453	466	12			
454	121	0		2	
455	385	5			
456	321	6			
457	854	22			
458	150	2			
459	401	8	2		
460	403	14			
461	420	5			
462	203	5	1		
463	302	8	2		
464	249	8			
465	287	6			
466	167	3			
467	400	10	2	2	
468	294	3			
469	111	0			
470	322	2			
471	309	8	1		
472	24	0			
473	141	0			
474	76	1			
475	122	4			

APPENDIX VI. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
476	393	11	1		
477	347	6			
478	105	2			
479	306	5			
480	318	0			
481	284	7			
482	252	9			
483	245	1			
484	52	0			
485	237	4		2	
486	265	7	1		
487	237	0			
488	212	5			
489	253	7			
490	325	6			
491	219	7			
492	260	2			
493	186	3			
494	217	1			
495	303	7			
496	146	1			
497	256	8	1		
498	257	3			
499	229	7			
500	365	3			

APPENDIX VII. Fruit crop parameters for the 263 terminal infructescences on one major branch of *Ateleia herbert-smithii* number 11 in 1985. All seed death was caused by *Apion johnschmitti*. Blanks are zeros. Note: branch numbers were assigned as the branches were taken from a large but nonrandomized branch pool and should be randomized before subsampling.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
1	284	9			
2	146	6	1	1	
3	47	2			
4	204	7	1		
5	120	5			
6	290	6	1		
7	140	2			
8	135	1	1		
9	382	4	1	1	1
10	173	4			
11	348	4			1
12	241	4			1
13	67	0			
14	282	7		1	

APPENDIX VII. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
15	60	2			
16	252	4	1		
17	93	0			
18	130	6			
19	60	0			
20	328	8	1		
21	123	3			
22	323	7			
23	80	4			
24	164	5			
25	163	5			
26	513	16			
27	213	4	1		
28	699	22	4		
29	302	6	4		
30	36	1	1		
31	255	13	2		
32	247	10	1		
33	141	6	1		
34	119	8	1		
35	221	7			
36	277	7	2		
37	206	1		1	
38	253	7	1		
39	275	10			
40	213	4	1		
41	339	7			
42	56	0			
43	73	1			1
44	43	3			
45	76	0			
46	253	7			
47	125	9			
48	197	3	1		
49	111	9			
50	160	7			
51	353	11			
52	148	1			
53	301	13			
54	67	1			
55	77	1	1		2
56	416	6	1		3
57	185	4			
58	202	9			2
59	202	5			
60	86	1			
61	342	6			
62	110	2			
63	89	2			
64	56	2			

Appendix VII. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
65	190	3			
66	40	0			
67	127	1			
68	128	2			
69	531	2	1	1	1
70	301	1			1
71	268	1	1		
72	609	16			1
73	280	5			
74	329	3	1		1
75	180	3			
76	556	9	1		1
77	131	3	1		
78	181	2			1
79	435	3			1
80	273	7			3
81	90	0			
82	453	7			1
83	350	7			2
84	95	3			
85	96	1			
86	357	2			2
87	144	8			
88	147	2			
89	516	2	1		1
90	46	0			
91	33	0			
92	133	2			
93	182	5			
94	172	6			
95	91	3			
96	35	1			
97	103	3			
98	211	7		1	
99	186	1			
100	135	2	1		
101	129	0	1		
102	206	3			
103	237	7			1
104	226	11			
105	34	3			
106	226	4			
107	116	1			
108	121	0			
109	294	12			1
110	70	0			
111	59	2			
112	141	2			
113	234	5	2		
114	158	2			1

Appendix VII. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
115	245	6		1	
116	275	9	2		
117	184	4	2		
118	35	1			
119	23	0			
120	292	5			
121	301	2			
122	98	3			
123	126	1			
124	33	0			
125	107	2			
126	229	2	1		
127	236	3			1
128	229	5	1		
129	37	2	2		
130	214	4			
131	68	6			
132	94	4			
133	79	0			
134	80	4			
135	242	8			
136	31	0			
137	40	2			
138	137	0	1		
139	257	7			
140	70	3			
141	101	2			
142	150	1		1	
143	134	4			
144	86	1			
145	52	0			
146	212	9			
147	234	2	1	1	
148	203	5			1
149	102	1			
150	392	10			2
151	209	4	1		
152	176	6			
153	41	1			
154	304	3			
155	43	0			
156	112	2			
157	81	3			
158	30	2			
159	135	4			1
160	333	4	2		2
161	38	2			
162	303	10			
163	217	5	3	1	
164	55	0			1

APPENDIX VII. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
165	253	8	1	1	
166	91	1			
167	93	1			1
168	252	1	1		
169	105	2			
170	105	0			
171	208	4			
172	160	3			
173	177	3			
174	55	2			
175	109	2			
176	49	1			
177	219	6			
178	115	4			
179	22	0			
180	97	4	2		
181	386	12	1		
182	168	7			
183	259	7		1	1
184	124	7			
185	66	3			
186	139	1	1		
187	88	0			
188	77	5			
189	129	2			1
190	354	6			
191	129	7			
192	138	4			
193	63	1	1		
194	29	3			1
195	167	3			
196	117	3		2	1
197	234	0			
198	128	7			1
199	172	4			
200	73	4			
201	123	3			
202	348	8			
203	343	13			2
204	82	0			
205	90	14			1
206	188	4			
207	185	5			
208	123	1			
209	94	2			
210	347	11			2
211	113	4			
212	68	2			
213	187	5			
214	77	1			1

Appendix VII. Continued.

Branch number	One-seeded fruits		Two-seeded fruits		
	Seed killed	Seed missed	One seed killed	None killed	Both killed
215	225	4			
216	126	3			
217	213	3			
218	214	4			1
219	159	2			
220	116	2			
221	173	1		1	
222	237	8			
223	96	7			
224	213	8		1	
225	47	3			
226	134	2	1		
227	183	2	2		1
228	210	3			1
229	370	9	1		
230	161	0			1
231	234	4	1		
232	529	13			
233	21	0			
234	94	2			
235	134	1			
236	121	4			
237	103	1			
238	65	3			
239	199	5			
240	91	7			
241	61	1			
242	208	7	1		
243	96	2			
244	251	6			
245	447	7			2
246	218	12			1
247	172	11			
248	513	8			
249	241	4			
250	64	0			
251	64	2			
252	154	3			
253	63	2			
254	402	2	2		1
255	271	7			
256	57	1			1
257	246	0			
258	309	3			
259	52	2			
260	41	0			
261	93	2			
262	71	0			1
263	217	6			1

ECOLOGICAL SUCCESS IN RELATION TO PLANT FORM AND FUNCTION IN THE WOODY LEGUMES

Philip W. Rundel[1]

Abstract

The legume family may well be considered the most ecologically successful family of flowering plants. Woody legumes are consistently an important component of canopy trees in lowland tropical forests throughout the world. This significant level of dominance extends to tropical seasonal forests, thorn scrub, and many warm desert ecosystems. The dominance of legumes as a whole and woody legumes in particular drops off sharply in temperate and boreal ecosystems; woody legumes are totally absent from the latter. In these temperate habitats, there is a notable correlation of legumes with disturbance or early successional progressions. Flexible adaptive responses over a wide range of ecosystems is the key to evolutionary and ecological success of the legumes. Adaptive variations are present in structural traits (growth-form, canopy architecture, root architecture, and leaf morphology) as well as in physiological traits (phenological control, solar tracking, tissue water relations, nitrogen fixation, and mycorrhizal symbionts). None of these traits is specific to legumes, but no other flowering plant family exhibits such a tremendous breadth of adaptive responses.

I. Introduction

The legume family is arguably the most ecologically successful group of flowering plants in the world. Legumes occur in virtually every terrestrial ecosystem, ranging from 80-m giants in the dipterocarp forests of southeast Asia to tiny desert ephemerals with a life span as brief as six weeks. The success of the legumes is measured both in terms of dominance and diversity. The legume family comprises more than 17,000 species, or approximately 7% of the described vascular plants of the world. Thus, it is exceeded in absolute species diversity by only the Compositae (Asteraceae) and Orchidaceae.

This paper presents a broad overview of the morphological and physiological adaptations of woody legumes that allow these taxa to be so ecologically successful in terms of dominance and productivity. The organization of this review is focused on three questions:1) In what ecosystems are woody legumes successful? 2) How ecologically successful are woody legumes? 3) What aspects of their physiological ecology make woody legumes successful? In answering these questions, particularly the latter, I will argue that the ecological success of woody legumes is a result of their evolutionary flexibility in adaptive response. The morphological and physiological adaptations utilized by legumes are not unique but are nevertheless impressively diverse for a single family.

II. Distribution and Ecological Success of Woody Legumes

Woody legumes are abundant and frequently dominant in ecosystems that span the full range of conditions of precipitation and length of dry season. These range from evergreen and semi-evergreen rainforest through humid and dry monsoon forests to savanna and warm deserts (Fig. 1). All of these ecosystems have evolved from tropical floristic elements. The abundance and dominance of woody legumes sharply declines in temperate and boreal ecosystems. As described below,

[1]Laboratory of Biomedical and Environmental Biology and Department of Biology, University of California, Los Angeles, California 90024, U.S.A.

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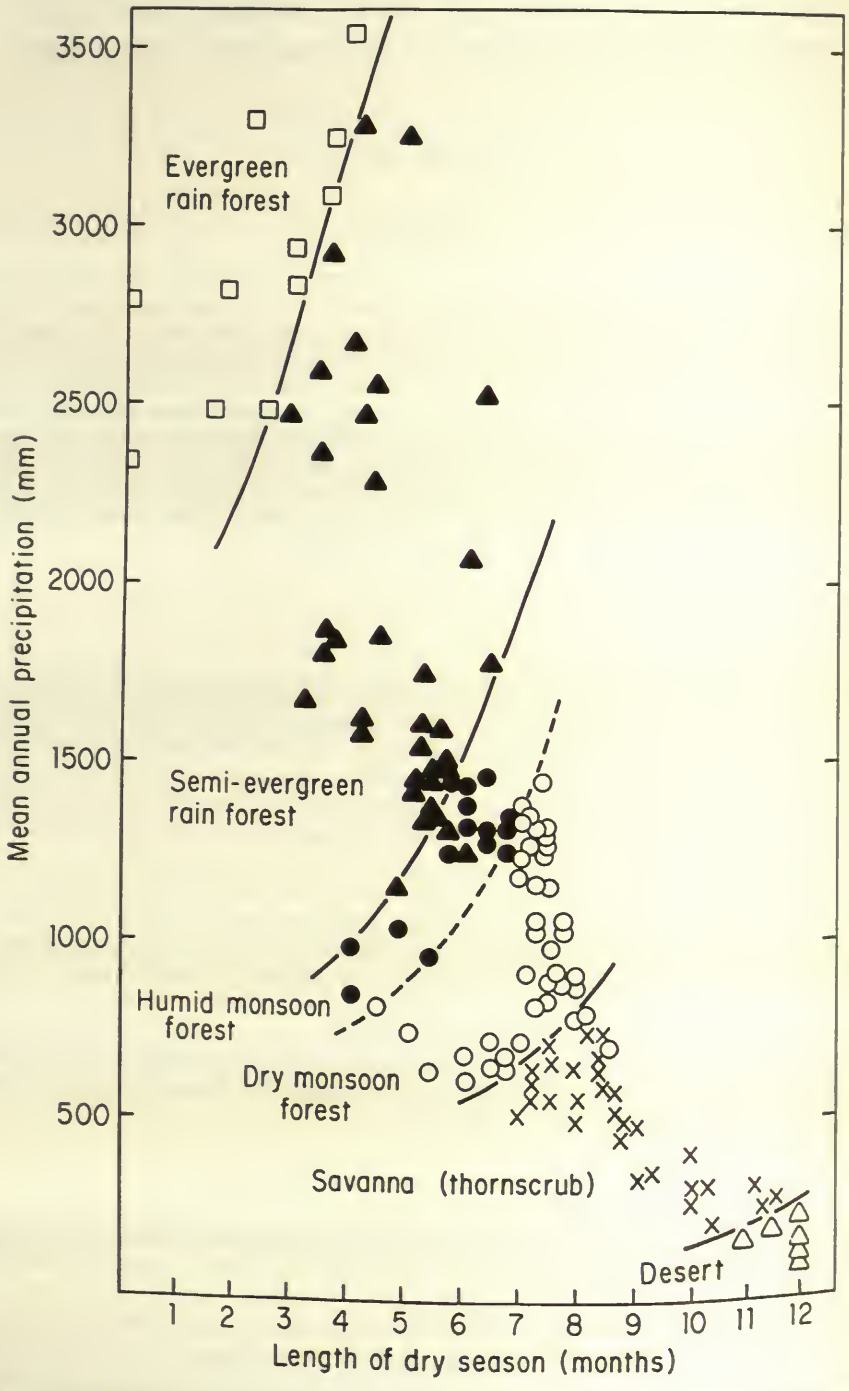


FIGURE 1. Distribution of tropical community types in relation to mean annual precipitation and length of dry season.

legumes in temperate ecosystems become increasingly dominated by herbaceous growth forms and increasingly restricted to successional habitats.

WET TROPICS

There are a variety of measures of ecological success for woody legumes. Two examples of this are shown in Figures 2 and 3 with comparative data on the relative frequency of legume species and legume stems from evergreen and semi-evergreen rainforests. In dipterocarp forests of southeast Asia, legumes comprise 3–5% of the total tree flora (Anderson, 1963; Ashton, 1964; Poore, 1968). In the dipterocarp forest of the Jengka Forest Reserve in Malaysia, legumes, as a group, comprised only the sixth most important family of canopy tree species, exceeded in order of diversity by the Dipterocarpaceae, Euphorbiaceae, Myrtaceae, Burseraceae, Myristicaceae, and Annonaceae (Poore, 1968). In low kerangas forests of Brunei, growing on nutrient-poor spodosols, legumes comprise 5.9% of tree and shrub species (Brunig, 1974).

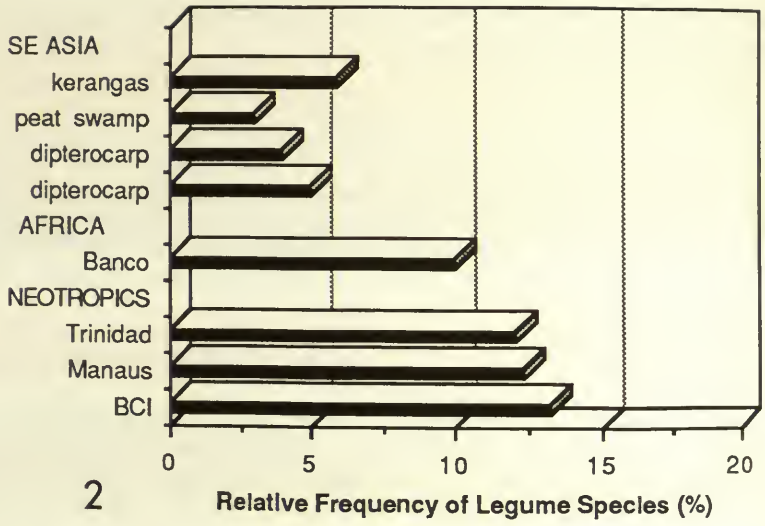
In the Neotropics, the frequency of legumes in the tree flora is dramatically higher than in southeast Asia, with typical values of 12–13% (Fig. 2). The frequencies are remarkably consistent in published data for this region (Beard, 1946; Prance et al., 1976; Fittkau & Klinge, 1973; Croat, 1978). Bernard-Reversat et al. (1978) reported intermediate relative species frequencies for woody legumes in central Africa.

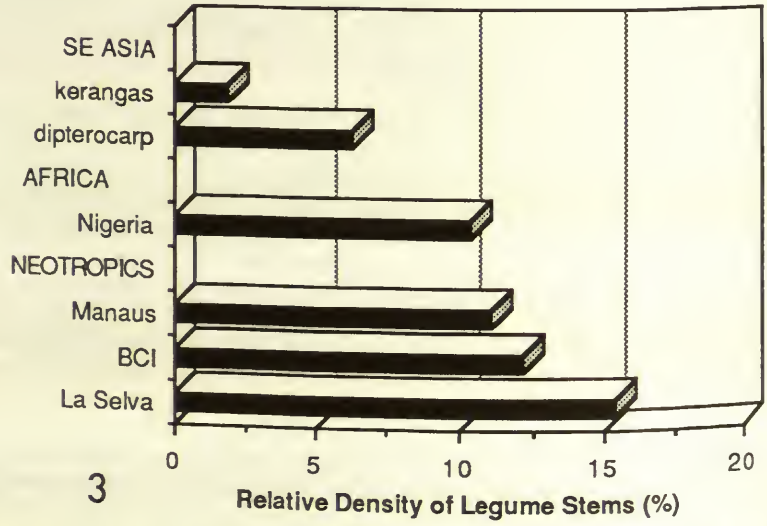
If legume success is measured in terms of the frequency of tree stems in wet tropical forests (Fig. 3), the geographic pattern is very similar to that described for species frequency. Legume trees comprise 6.3% of the total canopy tree stems and 8.0% of the basal area of canopy trees in the Jengka Forest Reserve (Poore, 1968). These values make the legumes the third most important family at this site both in terms of relative frequency of stems and relative basal area. Thus, the ecological success of the woody legumes is somewhat greater in terms of these measures than in terms of species diversity. One very large legume tree, *Koompasia malaccensis,* competed very well with the dominant dipterocarps and was the fourth most important tree species at this site (Poore, 1968). In the low nutrient kerangas forests, woody legumes comprise only 1.9% of the stems and 2.4% of the basal area, both lower values than that for relative species diversity (Brunig, 1974).

In neotropical rainforests, the relative frequency of legume tree stems (Fig. 3) and relative basal area of legumes is again much higher. At Manaus in the Amazon Basin, legume trees make up 11.1% of the forest stems and 11.2% of the forest basal area (Prance et al., 1976). On Barro Colorado Island and La Selva (Costa Rica), legumes respectively comprise 12.2% and 15.4% of the woody stems (Hubbell & Foster, unpublished data; Hartshorn, 1975). For central Africa, the relative frequency of legume tree stems is generally intermediate between those of southeast Asia and the Neotropics. At the Akure Forest Reserve in Nigeria, for example, legumes make up 10.4% of the tree stems (Lawton, 1978).

While legume densities of 12–15% of total tree stems are typical in lowland rainforests in the Neotropics, there are a number of examples of wet forests where legume densities may be considerably higher. In most cases, high levels of legume dominance are associated with the ecological success of individual species. *Pentaclethra macroloba* comprised 15.4% of the stems in the high-diversity forest at La Selva (Hartshorn, 1975). Swamp forests comprising virtual monocultures of either *Prioria copaifera* or *Pterocarpus officinalis* are widespread in the Caribbean area (Beard, 1946; Croat, 1978; Golley et al., 1975). A single species, *Mora excelsa,* forms 85–95% of the canopy trees in the wet Mora forests of Trinidad (Beard, 1946).

Perhaps the best documented example of changing legume dominance along relatively short environmental forest gradients comes from classic studies in British Guiana by Davis & Richards (1934). In Mora forests on lowland soils flooded during the wet season, legumes make up 16.9% of the tree species (Fig. 4), but 59% of the tree stems over 10 cm dbh. Nearly 40% of the total legume stems are *Mora excelsa.* The lower slope of small hills in this region support a morabukea





FIGURES 2, 3.—2. Relative frequency of legume species among trees in tropical raintorests. Data from Brunig (1974), Anderson (1963), Ashton (1964), Poore (1968), Bernhard-Reversat et al. (1978), Beard (1946), Prance et al. (1976), Fittkau & Klinge (1973), and Croat (1978).—3. Relative density of legume stems among trees in tropical rainforests. Data from Brunig (1974), Poore (1968), Lawton (1978), Prance et al. (1976), Hubbell & Foster (unpublished data), and Hartshorn (1975).

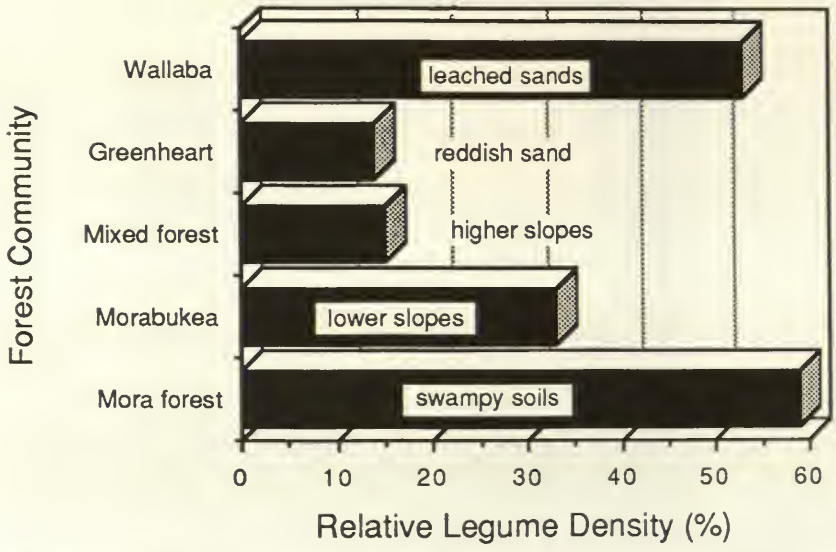


FIGURE 4. Relative density of legume stems among trees in tropical evergreen forest communities of British Guiana (Davis & Richards, 1934).

forest where 11.8% of the species and 33% of the tree stems are legumes. Most of this legume dominance is again due to a single species, *M. gonggrijpii*. Two intermediate forest types, the mixed forest (higher, better drained slopes) and greenheart forest (reddish, sandy soils) exhibit fairly typical neotropical values for relative frequency of legumes among tree stems, with 15% and 14% respectively. At the other end of the gradient, on porous leached sands, the Wallaba forest has 53% legume stems. As with the mora forest, 40% of the legume coverage is due to a single species, in this case *Eperua falcata*. Thus, along this gradient legume dominance is typical of other neotropical lowland rainforests in intermediate habitats, but up to four times higher at the extremes of flooded and highly leached soils.

Although woody legumes form a major component of forests in the low nutrient Wallaba forest of British Guiana, they form a relatively small part of stem or basal area cover in the extremely low nutrient campina forests of the Amazon Basin (Anderson et al., 1975). This parallels the low relative dominance described above for the structurally similar kerangas forests from Sarawak and Brunei. Similarly, legume dominance drops rapidly with increasing elevation from lowland rainforests in the Neotropics. Montane rainforests and cloud forests in Central America, South America, and the Caribbean have a very low relative dominance of woody legumes (Lawton & Dryer, 1980; Sugden, 1982; Howard, 1968).

The distribution of legume species within the floras of lowland tropical rainforests is strongly biased toward woody growth forms, even more so than is the general pattern of the total forest flora. This can be illustrated with data from the seasonal evergreen rainforest of Barro Colorado Island, Panama (Croat, 1978). Sixteen percent of the total flora are canopy trees, compared to 40% of the legumes (Fig. 5). Legumes are also overrepresented among lianas and vines in the flora. In contrast, legumes are strongly underrepresented among understory trees (treelets), shrubs, and herbs. In the kerangas forests of Sarawak and Brunei, 67% of legumes are trees, compared with 69% for all species (Fig. 6). Legumes are overrepresented in shrub and vine growth forms, the latter strongly. None of the 96 species of herbaceous plants in these forests are legumes (Brunig, 1974).

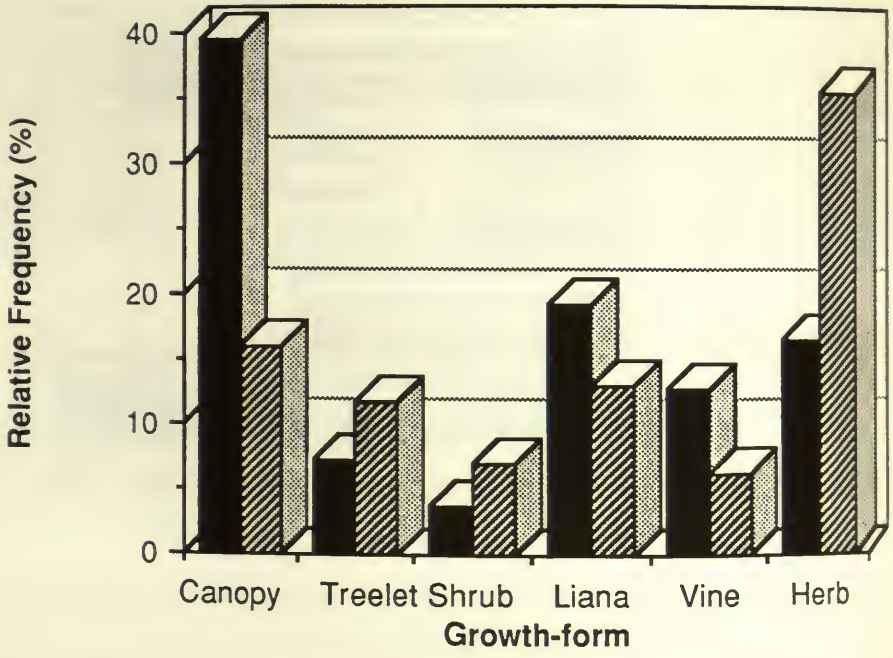


FIGURE 5. Relative frequency distribution of growth-forms for legumes (solid bars) and the total flora (diagonal bars) on Barro Colorado Island, Panama. Data from Croat (1978).

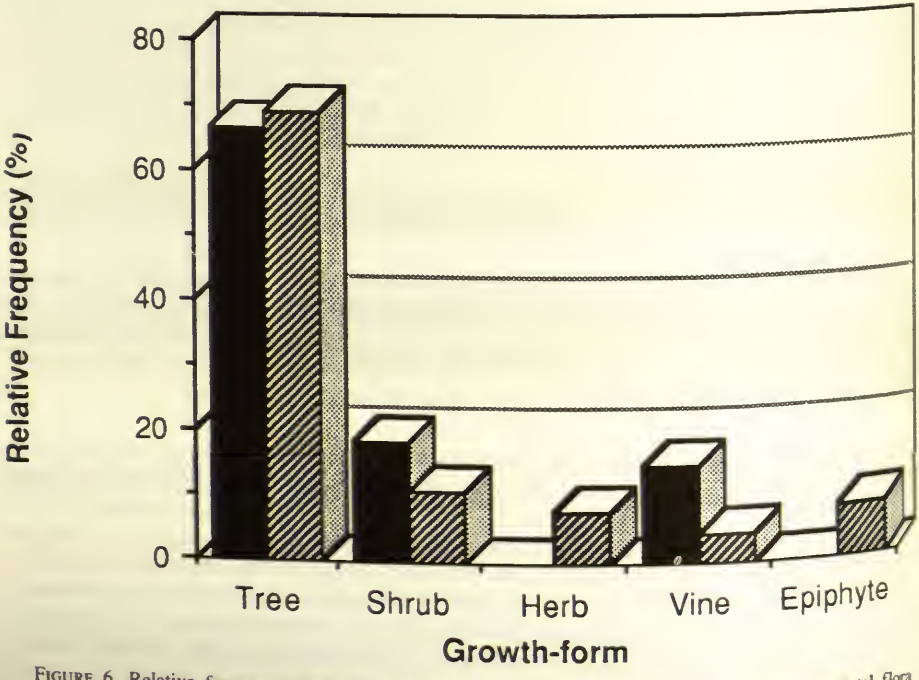


FIGURE 6. Relative frequency distribution of growth-forms for legumes (solid bars) and the total flora (diagonal bars) for kerangas forests in Sarawak and Brunei. Data from Brunig (1974).

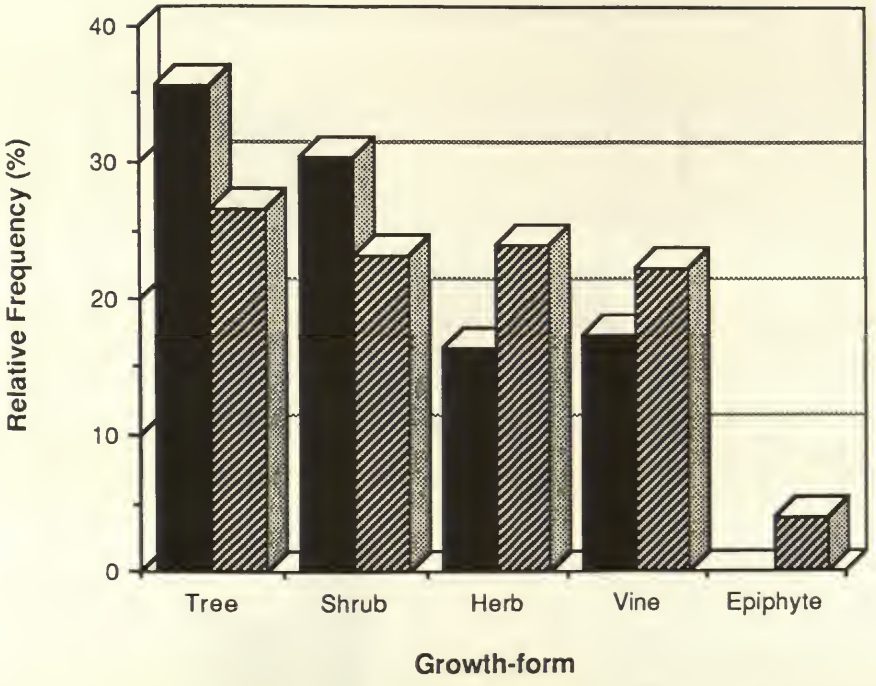


FIGURE 7. Relative frequency distribution of growth-forms for legumes (solid bars) and the total flora (diagonal bars) for seasonal forests at Chamela, Mexico. Data from Bullock (1985).

SEASONAL TROPICS AND DESERTS

The tendency toward ecological success of woody plant growth forms in legumes in tropical ecosystems applies to seasonal tropical forests as well. In the dry forests at Chamela on the west coast of Mexico, where 16% of the total flora are legumes (Bullock, 1985), trees and shrubs respectively comprise 27% and 23% of the total flora, but a significantly higher 36% and 30% of the legumes (Fig. 7). Legumes are underrepresented as herbs, vines, and epiphytes. They have a similar floristic diversity of 18.6% of the total flora in the dry Miombo woodlands of Africa (Malaisse, 1978). They also form an important floristic element in the cerrado vegetation of Brazil (Eiten, 1972).

Although quantitative data appropriate for comparative analyses are lacking, woody legumes enjoy perhaps their greatest relative ecological success in subtropical thorn forests of the world. In Africa and Australia, species of *Acacia* are the overwhelming dominants in these communities (Palgrave, 1977; Ullmann, 1985; Hopper & Maslin, 1978; Whibley, 1980), while in North and South America, woody legumes may make up 90% or more of the woody plant cover in thornscrub, most notably with species of *Prosopis* and *Acacia* (e.g., Gentry, 1942).

Deep Canyon in the Sonoran Desert of California has a legume flora with trees and shrubs each comprising 13% of all legumes (Fig. 8a). Annual legumes make up 26% of this total, with herbaceous perennials being the most abundant legume growth-form (Zabriskie, 1979). In the higher Mojave Desert of south-central Nevada, where winter temperatures are much lower, trees and shrubs each comprise less than 6% of the total legume flora (Fig. 8b). Herbaceous growth forms are strongly dominant, with herbaceous perennials and annuals respectively comprising 53% and 33% of the total flora (Beatley, 1976). The shift toward herbaceous growth forms is even more

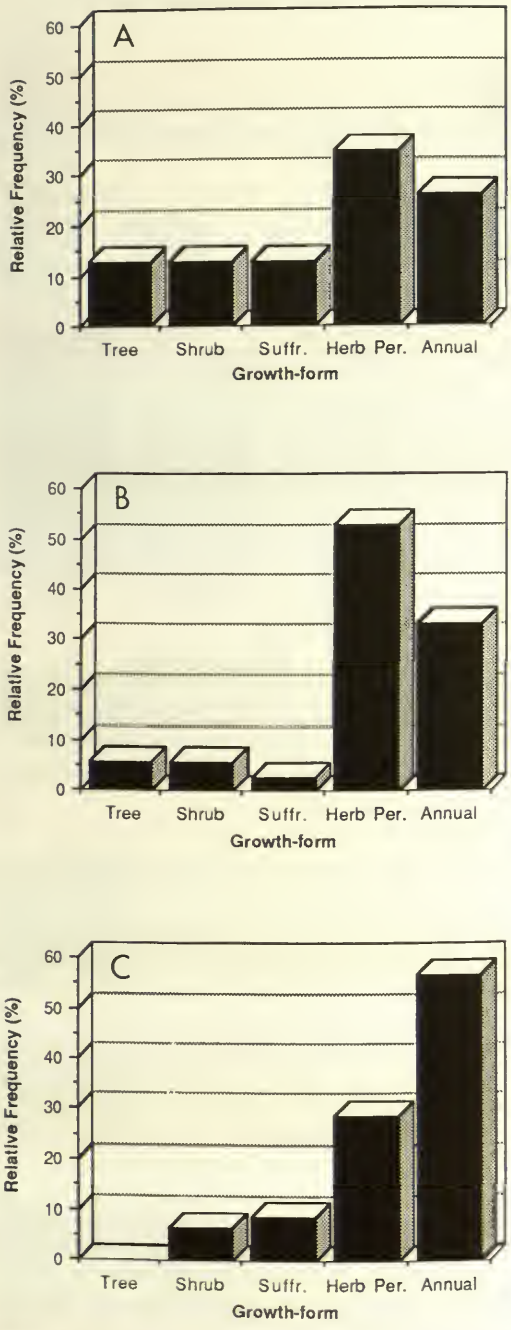


FIGURE 8. Relative frequency of growth-forms of legumes in warm desert and chaparral ecosystems.—A. Deep Canyon, Sonoran Desert scrub (Zabriskie, 1979).—B. South-central Nevada–Mojave Desert scrub (Beatley, 1976).—C. Santa Ana Mountains, California (Lathrop & Thorne, 1978).

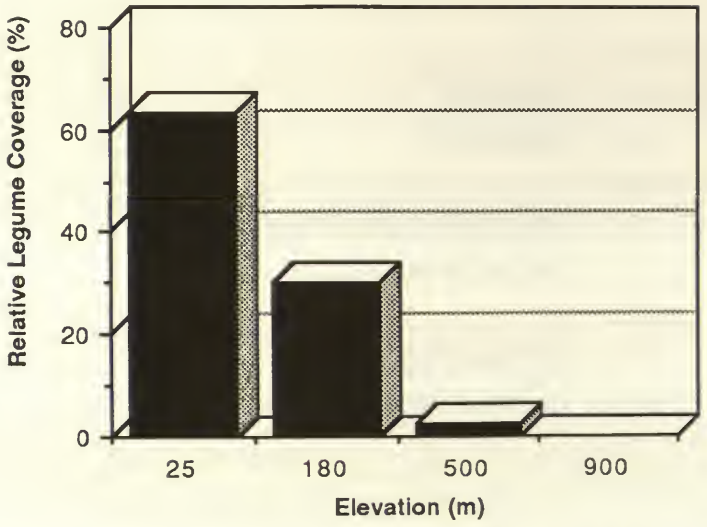


FIGURE 9. Change in relative dominance of woody legumes with elevation at the Deep Canyon Reserve in the Sonoran Desert of California. Data from Zabriskie (1979).

dramatic in the chaparral of southern California. In the Santa Ana Mountains near Los Angeles, no tree legumes are present and only 6% of the total legumes are shrubs (Fig. 8c). Here annuals comprise 57% of the growth-form for legumes (Lathrop & Thorne, 1978).

The impact of relatively small environmental gradients on legume dominance in southern California can be seen in an elevation gradient of only 900 m at Deep Canyon (Fig. 9). On the valley floor at 25 m elevation, woody legumes make up 64% of the total perennial vegetation cover (Zabriskie, 1979). On alluvial plains at 180 m, relative legume coverage drops to 30%.

Compared to the global average frequency of 7% for legume species, warm desert ecosystems are particularly rich in legumes (Fig. 10). The Sonoran Desert has 11.6% legume flora, with woody growth forms richly represented (Shreve & Wiggins, 1964). In central Australia, legumes comprise 13% of the flora (Jessop, 1981), and three-fourths of these are woody shrubs and tree species. The floristic diversity of legumes in the Sahara Desert is similarly high (Ozenda, 1958).

TEMPERATE COMMUNITIES

Mediterranean-type ecosystems have legume diversities very similar to the global average. For fynbos and related communities in South Africa, legumes comprise 7.3% of the flora of the Cape Peninsula (Levyns, 1966), 7.3% of the rich flora of the nearby Cape Hangklip area (Boucher, 1977), and 10.9% of the unique fynbos area overall (Bond & Goldblatt, 1984). Woody shrubs are not an important component of legume floras in the fynbos, although semiwoody species of *Aspalathus* (Kruger, 1979), *Lebeckia, Psoralea,* and *Otholobium* (Stirton, pers. comm.) may be locally important dominants after fires. In California chaparral and related woodland communities, legumes comprise 6–7% of the total flora. Values are remarkably consistent with 6.5% in the Santa Cruz Mountains near San Francisco (Thomas, 1961), 6.5% in San Luis Obispo County in the central coast (Hoover, 1970), and 6.9% in the Santa Ana Mountains of southern California (Lathrop & Thorne, 1978). The diversity of woody legumes in four of the five Mediterranean regions is relatively low at both the species and genus level. This is not true of the Mediterranean region of

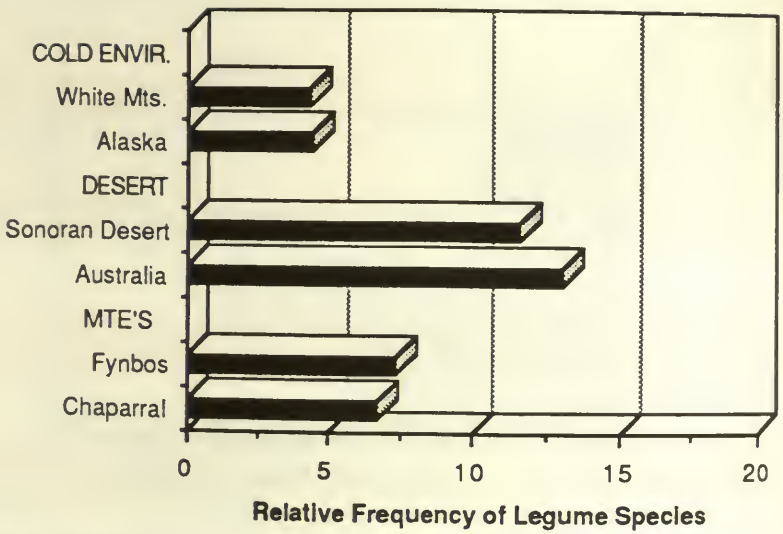


FIGURE 10. Relative frequency of legume species in the floras of temperate and arid shrublands. Data from Lloyd & Mitchell (1973), Hulten (1968), Shreve & Wiggins (1964), Boucher (1977), Levyns (1966), Lathrop & Thorne (1978), Hoover (1970), and Thomas (1961).

TABLE 1. Changes in dominant growth-forms and leaf phenology for dominant legumes along a gradient from mesic tropical forests to arid deserts and mediterranean scrub ecosystems.

	Growth-form	Leaf phenology
Tropical forest	trees	evergreen
	lianas	semi-evergreen
Seasonal forest	trees	drought-deciduous
	shrubs	
Thorn forest	low trees	drought-deciduous
	shrubs	
Warm desert	shrubs	drought-deciduous
	low trees	winter deciduous
	herbs	aphyllous
Mediterranean scrub	shrubs	drought-deciduous
	herbs	aphyllous

Europe and North Africa, however, where diverse assemblages of woody legumes have evolved (Polunin & Smythies, 1973).

Cold temperate environments typically have a relatively low diversity of legumes, and woody shrubs and trees are rare to totally absent (Fig. 10). Eastern deciduous forests in the United States contain notable examples of woody legumes (e.g., *Gleditsia* and *Robinia*), but these taxa are predominantly early successional elements and are unimportant or absent in climax forest stands. In the cold and dry White Mountains of California, only 4.3% of the flora are legumes, and only annual herbaceous perennial species are present (Lloyd & Mitchell, 1973). The flora of Alaska includes a similar 4.4% legumes, also all herbaceous, with a low proportion of annuals (Hulten, 1968).

When gradients in legume growth-form and phenology are viewed from wet tropical forests to dry

deserts and mediterranean scrub, there are clear patterns of change (Table 1). At the mesic end of the gradient, legumes are predominantly trees and woody lianas, with evergreen or semi-evergreen leaf phenology. With increasing drought interactions, the dominant growth-forms become reduced in stature, and leaves become reduced in size and have lowered longevity. Growth-forms change first to lower stature trees and shrubs, and finally to domination by low shrubs and herbs. In more temperate environments, herbaceous perennials become the dominant growth-form. With increased seasonality and associated drought stress, the dominant leaf phenology becomes drought-deciduousness. Aphyllous growth-forms with stem photosynthesis become important elements in legume floras from the driest end of the gradient.

III. Physiological Adaptations

Are woody legumes different from woody plants in other families? Do they demonstrate unique characteristics of photosynthetic capacity, water relations, or nutrient relations that allow them to attain their obvious degree of ecological success in competition with other groups of plants? The answer to these questions is clearly no. Legumes certainly possess no physiological attributes that are not present in other plant groups. As a family, however, the legumes exhibit a broader suite of adaptive traits than virtually any other family. It is this evolutionary flexibility that has allowed the tremendous ecological success of woody legumes.

Rather than dwell on a comparison of the physiological ecology of woody legumes with that of other plant groups, it seems most appropriate in this review to illustrate the range of adaptive traits, both physiological and anatomical, that differentiate woody legumes at the extremes of an environmental gradient from tropical lowland rainforest to warm desert ecosystems.

Photosynthetic Capacity

The photosynthetic capacity of leaf tissues of woody legumes from the desert and mediterranean climate areas of California are equal to those of agricultural legume crops grown under nonlimiting water conditions (Fig. 11). For example, *Prosopis glandulosa,* a common phreatophytic shrub or tree that has moderately scherophyllous leaflets and grows in the southwestern deserts of North America, has been shown to have a photosynthetic capacity greater than 30 $\mu mol/m^2/sec$. (Mooney & Gulmon, 1979). An annual desert legume, *Lupinus arizonicus,* has a photosynthetic capacity of 50 $\mu mol/m^2/sec$. (Forseth & Ehleringer, 1983). Clearly, therefore, adaptation to drought stress does not necessarily require tradeoffs in photosynthetic capacity per unit of leaf area during periods of the year when leaves are present. In *Lotus scoparius* and *Cercidium floridum,* that period is generally only a few months, but in *Prosopis glandulosa* it is 11 months.

There are no recent field data published on the photosynthetic capacity of tropical rainforest legumes. Phytotron studies of seedlings of *Dipteryx panamensis* and *Pentaclethra macroloba* from Costa Rica reported maximum photosynthetic rates of only 6–8 $\mu mol/m^2/sec$. (Oberbauer & Strain, 1984). If these data are applicable to field plants, they would suggest an unexpected limitation of enzyme capacity for carboxylation.

While photosynthetic capacity is a useful instantaneous measure, ecological success is based much more on optimizing rates of carbon fixation over much longer periods of time. In environments where solar irradiance is high and water stress may be significant, leaf orientation is an important factor in determining diurnal levels of carbon uptake (see Hebert, this volume). When leaf orientation exhibits a random azimuth, the mean diurnal cycle of incident quantum flux follows a bell-shaped distribution, with maximum irradiance at midday (Fig. 12). Many herbaceous legumes, as well as plants in many other families, have diaheliotrophic leaves which track the sun during the

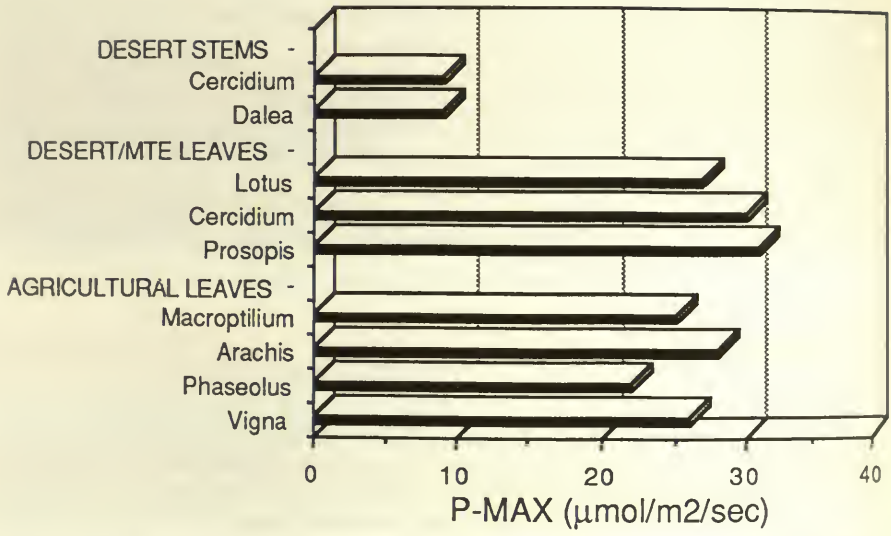


FIGURE 11. Photosynthetic capacity (µmol/m^2/sec.) for desert and agricultural legumes. Data from Rundel et al. (unpublished), Comstock & Ehleringer (1986), Mooney & Gulmon (1979), and El-Sharkawy et al. (1985).

day (Ehleringer & Forseth, 1980). Such leaves can increase their incident solar radiation up to 167% over those with a random azimuth. Paraheliotrophic leaves, which track to maintain a parallel leaf orientation to the sun, have a low incident quantum flux. This may represent a potential adaptation to drought conditions by reducing heat loads and minimizing transpirational fluxes. Many woody legumes utilize a strategy of leaf orientation combining aspects of both dia- and paraheliotrophic movement. Leaves track the sun perpendicularly in the morning and afternoon to increase levels of incident quantum flux, and track parallel at midday to reduce heat load. This mixed strategy of leaf orientation has been carefully documented in kudzu *(Pueraria lobata)* in the eastern United States (Forseth & Teramura, 1986) and appears to be common in woody legumes from temperate to tropical habitats.

Many deciduous woody legumes have chlorophyllous tissues in their twigs, permitting continued stem photosynthesis at those times of year when leaves are absent. This condition is most apparent in desert and thornscrub legumes, but it occurs in tropical communities as well. While the absolute photosynthetic capacity of stem photosynthesis in woody legumes is only about one-third that of leaf tissues (Fig. 11), the water use efficiency of this fixed carbon is high. The morphology and functionality of stem photosynthesis in woody legumes is very different from that of temperate forest trees with chlorophyllous stems (e.g., *Populus tremuloides*). The desert legumes lack lenticels, but stomata are abundant on the stem surface. Periderm formation on stems is delayed for several to many years in these taxa, allowing stomatal control over photosynthesis and transpiration. The anatomical correlates of stem photosynthesis in desert legumes show a remarkable convergence with those in many other types of stem-photosynthetic desert shrubs (Gibson, 1983; Fig. 13). A multiple-layered epidermis found in two common woody legumes, *Cercidium microphyllum* and *Hoffmannseggia microphylla*, closely parallels that of *Asclepias subulata* (Asclepiadaceae) and *Castelia emoryi* (Simaroubaceae). A one-layered epidermis with uni- or multicerate trichomes occurs in the legumes *Cassia [Senna] armata* and *Dalea spinosa* as well as in *Justicia californica* (Acanthaceae) and *Salazaria mexicana* (Labiatae).

Studies of stem photosynthesis in *Cercidium floridum* in the Sonoran Desert of California have

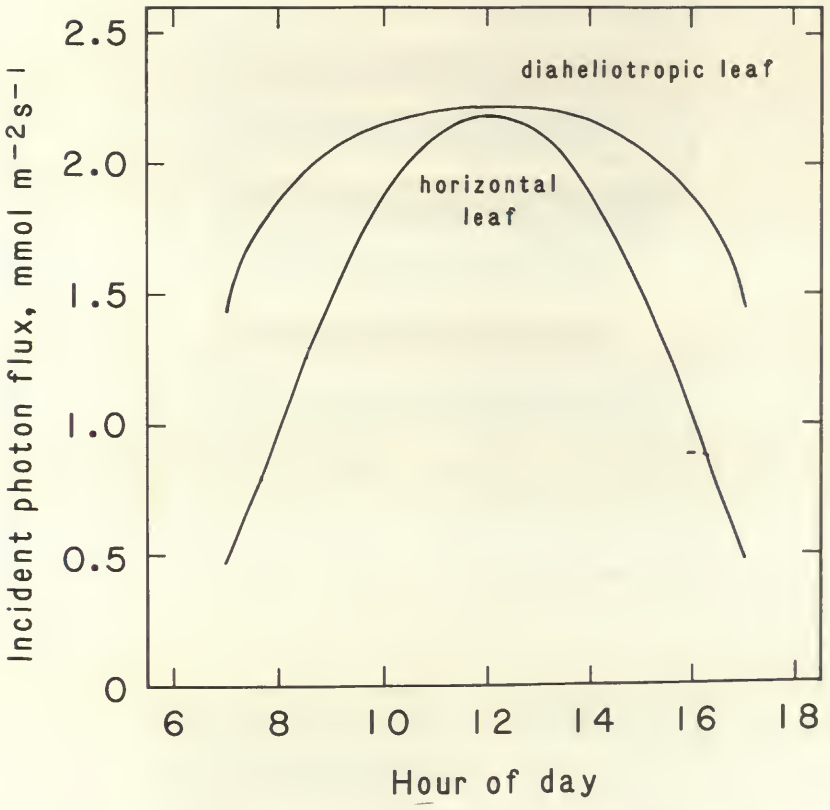


FIGURE 12. Diurnal pattern of incident quantum flux for horizontal and diaheliotropic (from Ehleringer & Forseth, 1980).

demonstrated the potential effectiveness of stem photosynthesis on even relatively large branches. Chlorophyll contents per unit surface area are not statistically different between smallest twigs and stems greater than 7.5 cm in diameter (Fig. 14). The relative photosynthetic capacity of these large stems was more than 50% that of the fine twigs (Szarek & Woodhouse, 1978).

A parallel strategy to stem photosynthesis in a number of legumes, most notably in the genus *Acacia*, is the evolution of photosynthetic phyllodes to replace leaf tissues. Phyllodes are flattened petioles that provide a highly sclerophyllous substitute for leaf tissues. As with stem photosynthesis, the photosynthetic capacity of phyllodes is relatively low, but the water use efficiency is relatively high. In Australia, there is a very dramatic gradient of dominance in *Acacia* morphology as one moves from more mesic forest areas where leafy acacias dominate to the central desert where phylodinous species of the genus are the overwhelming dominants. Hellmuth (1969, 1971) described the photosynthetic rates and transpiration of *Acacia* phyllodes in Australian deserts. Interestingly, phyllodes have not evolved in desert acacias in the African or New World deserts. Comparative dry season transpiration rates of leafy acacias have been described for Africa (Ullmann, 1985) and Australia. In the Hawaiian Islands, young *Acacia koa* has leaves, but in maturity these are replaced by phyllodes. As expected, the physiological characteristics of the leaves and phyllodes are quite different.

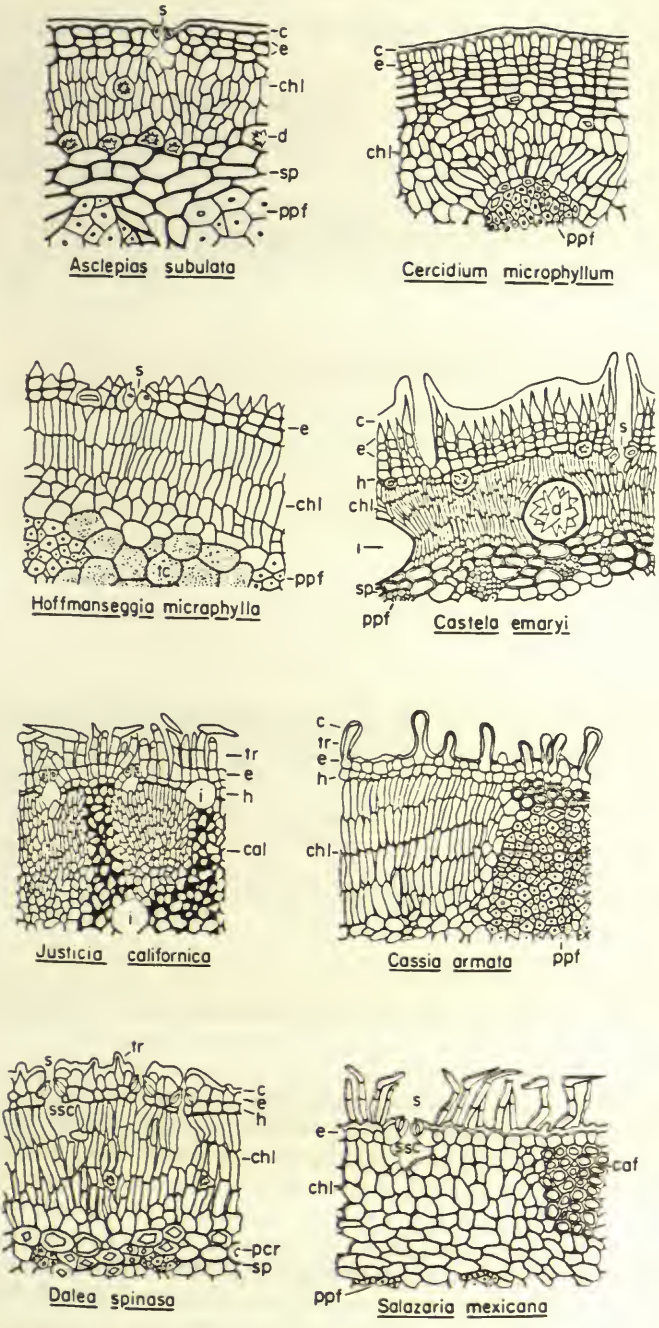
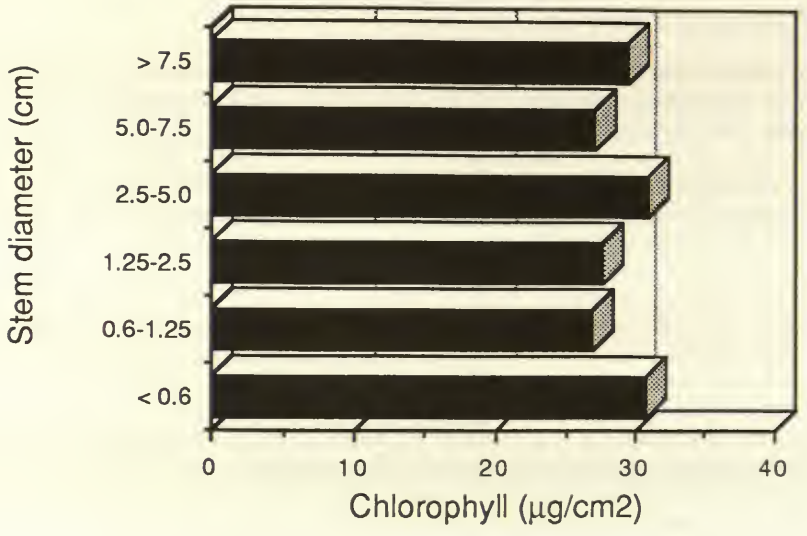


FIGURE 13. Anatomical structure of photosynthetic stems of aphyllous desert shrubs and trees (Gibson, 1983). c = cuticle, chl = chlorenchyma, cof = cortical fibers, col = collenchyma, d = druse, e = epidermis, h = hypodermis, i = idioblastic secretary cell, pcr = prismatic crystal, ppf = primary phloem fibers, s = stomate, sp = sclerified parenchyma, ssc = substomatal chamber, tc = tannin cells, tr = trichome.



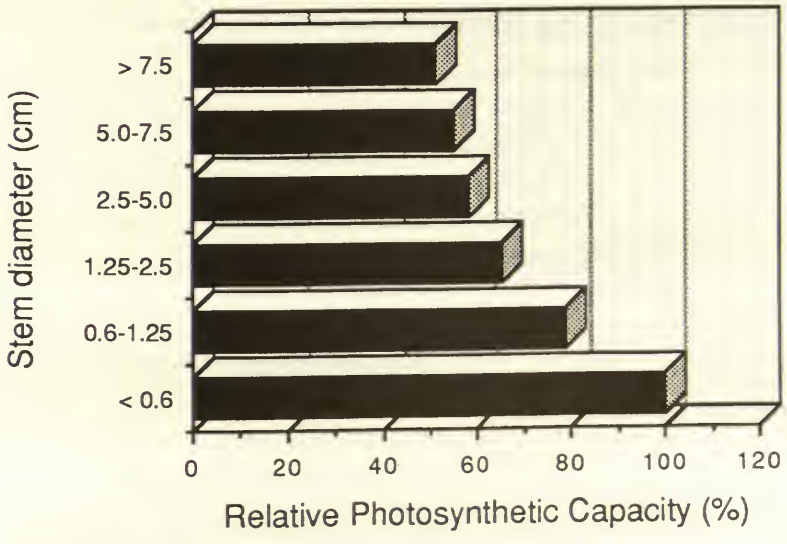


FIGURE 14. Chlorophyll content and relative photosynthetic capacity of twigs of different stem diameters in *Cercidium floridum*. Data from Szarek & Woodhouse (1978).

WATER RELATIONS

Since gradients from lowland tropical rainforests to warm desert ecosystems involve significant changes in environmental water stress, it is interesting to look at how woody legumes have evolved differences in the physiological response to them. The soil water potential in the rooting zone of a plant is a function of the matric potential (i.e., capillarity effects), the solute potential (effects of solutes), and the gravitational potential. Since plants can only take up water from the soil along a passive potential gradient from high to low potentials (potentials are negative numbers), survival in arid regions requires that plants do one of two things to maintain positive rates of water uptake. One of these is to utilize deep roots to tap pools of groundwater, and many woody desert legumes have adopted this phreatophytic strategy. Roots of *Prosopis glandulosa* have been reported to reach depths of more than 50 m (Phillips, 1963), and rooting depths of 5 m or more are common (Rundel et al., 1982; Mooney et al., 1980). Again, this is not a unique strategy of legumes. The second available strategy is to maintain plant water potentials lower than those of the soil. An important means of accomplishing this is the utilization of cellular osmotica to allow tissues to develop and maintain a favorable gradient in water potential from leaf tissues to soil. Both organic and inorganic solutes are important aspects of drought stress in most desert plants.

Under full turgor conditions, the osmotic potential of desert legumes is surprisingly similar to that of saplings of tropical forest legumes. For example, *Prioria copaifera*, a common swamp species in Panama, has an osmotic potential of −1.9 MPa, compared with values of −2.0 to −2.6 MPa in a range of desert legumes (Fig. 15). The nature of adaptation to water stress can be seen more clearly, however, by looking at conditions at the point where zero cell turgor is reached. At zero turgor no growth is possible, and lower levels of water potential commonly lead to permanent tissue damage. Thus, a relatively low osmotic potential at zero turgor would be expected to correlate with the ability of a species to maintain net growth at increasing levels of water stress. The small differences in osmotic potential at full turgor between tropical forest and desert legumes are greatly magnified at zero turgor (Fig. 15). Desert legumes reach this point in summer at osmotic potentials of −2.7 to −4.2 MPa, compared with levels of −1.4 to −2.4 in the tropical forest legumes. As might be expected, since the metabolic costs of maintaining osmoticum are relatively high, desert legumes have a large seasonal change in the value of osmotic potential. Under conditions of minimal water stress, in winter fewer osmotica are utilized and osmotic potentials are relatively high compared with the lower potentials reached under summer conditions (Nilsen et al., 1983, 1984). Tropical forest legumes exhibit little or no significant change in their seasonal osmotic relations (Rundel, unpublished data).

The pattern of observed differences between osmotic potentials at full and zero turgor is largely a function of the relative amount of water that can be lost from leaf tissues before the point of zero turgor is reached. There is a striking difference between the water deficit at zero turgor in desert and tropical forest legumes (Fig. 16). Desert legumes can lose 26–30% of their leaf water before reaching this point (Nilsen et al., 1983, 1984), compared with 12–21% in the four tropical legumes studied (Rundel, unpublished data).

NUTRIENT RELATIONSHIPS

Under conditions where water is not a limiting resource, nitrogen availability is a critical factor in controlling potential growth and production in woody plants. Soils with limited nitrogen availability are widespread in both arid and moist tropical ecosystems. The presence of nitrogen-fixing root nodules in many legumes is clearly an important aspect of their ecological success. The nature of biological nitrogen fixation is discussed in considerable detail in other papers in this volume.

The importance of nitrogen fixation in woody desert legumes has been clearly demonstrated. Phreatophytic species such as *Prosopis glandulosa* are able to maintain unexpectedly high levels of

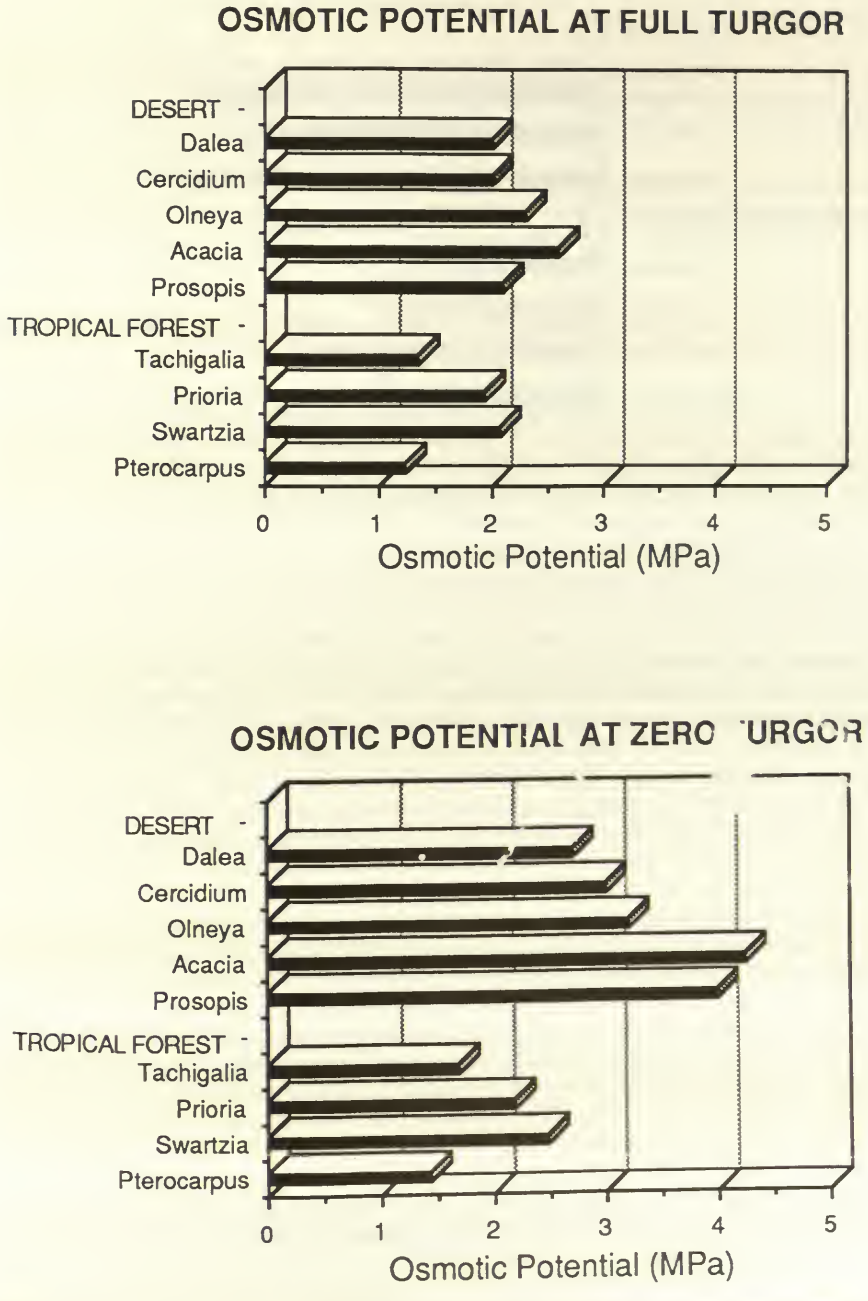


FIGURE 15. Osmotic potentials at full and zero turgor in woody legumes from desert and tropical forest habitats. Data from Nilsen et al. (1983, 1984), Meinzer & Rundel (unpublished), and Rundel (unpublished).

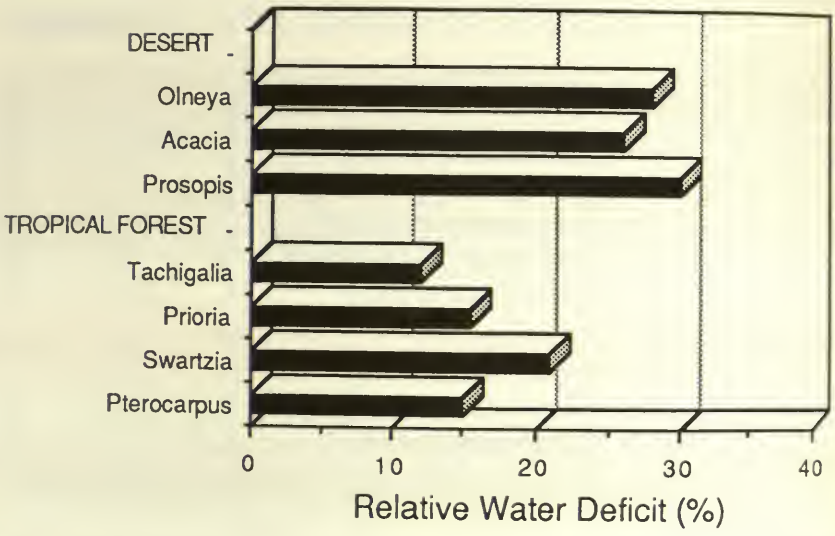


FIGURE 16. Relative water deficit at zero turgor in woody legumes from desert and tropical forest habitats. Data from Nilsen et al. (1983, 1984) and Rundel (unpublished).

net primary production by decoupling themselves from the normal limiting factors of water and nitrogen availability (Rundel et al., 1982; Sharifi et al., 1982). Approximately 60% of the net nitrogen increment each year in *P. glandulosa* comes from symbiotic fixation (Shearer et al., 1983). In another small desert tree, *Dalea spinosa*, approximately 30% of the nitrogen is fixed, while in herbaceous desert legumes 80–95% of the nitrogen can be fixed.

The ready availability of nitrogen is important in allowing increased levels of productivity, and nitrogen is an essential component of many types of secondary plant compounds, most notably the alkaloids and nonprotein amino acids—compounds that are a significant factor in the evolution of chemical defenses against herbivory in the legumes.

The maintenance of symbiotic associations to fix nitrogen requires a significant metabolic expense. This suggests a competitive disadvantage to legumes under conditions where nitrogen is not a limiting factor or where carbon is limiting. In temperate ecosystems, this disadvantage is readily seen in the successional role of many legumes. Legumes, as well as members of other plant families that symbiotically fix nitrogen, are typically most dominant in early stages of primary or secondary succession where nitrogen may be limiting. As succession proceeds to more mature communities, legumes generally become much reduced in importance, particularly in shaded forest understories where light is the primary limiting factor. The competitive relationships of herbaceous legumes and pasture grasses have been studied in detail (see Sackville Hamilton, this volume), and these studies confirm the expected pattern of competitive dominance by the legumes when nitrogen is limiting and by grasses when nitrogen is plentiful.

In tropical forest ecosystems where legumes are particularly important ecologically, there is no clear pattern of successional change in legume dominance through time. Woody legumes that fix nitrogen maintain a strong dominance despite closed canopy conditions. This suggests that these forest systems are much more dynamic than was once thought. Our current understanding of the importance of gap regeneration in tropical forests is consistent with the idea of small-scale mosaic of successional change.

Nitrogen fixation is clearly not a prerequisite for ecological success by legumes. Recent compilations by Allen & Allen (1981) demonstrate that 10% of the Mimosoideae, 70% of the Caesal-

pinioideae, and 2% of the Papilionoideae studied do not fix nitrogen. For example, *Cercidium floridum* and *Acacia greggii*, two successful woody legumes in the Sonoran Desert of California, do not fix nitrogen. In the Amazon Basin of South America, many legume trees that are well nodulated on the rich varzea soils of the floodplains lack nodules completely on the more widespread and nutrient-poor oxisols, apparently because of the strongly limited availability of phosphorus (Sylvester-Bradley et al., 1980).

Our ongoing studies of the physiological ecology of tropical forest trees on Barro Colorado Island, Panama, have revealed some puzzling data on the competitive ability of legume saplings in the forest understory. In this densely shaded habitat one would expect that nitrogen fixation would be a metabolic liability because of the limited ability to fix carbon. Furthermore, soils at this site that are derived from basalt parent material are relatively rich in nutrients. Nevertheless, data collected using the natural abundance ratios of ^{15}N (see Kohl & Shearer, this volume) strongly suggest that at least several species of legumes fix 20–35% of their tissue nitrogen (Rundel, unpublished data).

Phosphorus availability is clearly of importance to woody legumes. The importance is manifested in the well-developed associations of mycorrhizal fungi. The process of symbiotic nitrogen fixation in particular has a high requirement for phosphorus. As discussed by Alexander in this volume, a number of Caesalpinioideae are known to have ectomycorrhizal symbionts rather than the typical vesicular-arbuscular group of endomycorrhizal symbionts. The ecological significance of such symbionts is not clearly established, but there does appear to be a correlation of many known ectomycorrhizal species with swampy or riverine soils. These are the types of habitats where legumes are often overwhelmingly dominant. A clear exception to this is the genus *Brachystegia*, characteristic of well-drained savanna soils, which is reported to be ectomycorrhizal. Ectomycorrhizal fungi are typical of conifers but are relatively rare among angiosperms. The most notable groups of angiosperms with these symbionts are the Fagaceae and Dipterocarpaceae. It is tempting to associate the patterns of strong ecological dominance by the Pinaceae and these two families with the presence of ectomycorrhizae fungi, and to look for parallel situations among the legumes. To date, however, no one has been able to establish a clear causal linkage.

IV. Conclusions

There is no question that the legumes represent a remarkable evolutionary success story. This family unquestionably represents the most ecologically important family of woody plants in the world. Despite their relatively minor dominance in most temperate communities, legumes are major components of virtually all lowland tropical and subtropical forest systems in the world, as well as being the overwhelming dominants in many warm desert and thornscrub communities. No other family is an important dominant over such a broad range of ecological habitats.

The evolutionary and ecological success of the legumes over such a wide range of ecosystems is a function of their flexible adaptive responses. Remarkable levels of adaptive variation are present in structural traits such as growth-form, canopy architecture, root architecture, and leaf morphology, as well as physiological traits such as phenological controls, solar tracking, tissue water relations, nitrogen fixation, and mycorrhizal symbionts. While other successful families demonstrate similar adaptive flexibility for one or several such traits, none exhibits the tremendous breadth of adaptation described here for the legumes.

Acknowledgments

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References

Allen, O. N. & E. K. Allen. 1971. The Leguminosae: A Source Book of Characteristics, Uses and Nodulation. Univ. of Wisconsin Press, Madison, Wisconsin.

Anderson, A. B., G. T. Prance & B. W. P. de Albuquerque. 1975. Estudos sobre a vegetaçao das Campinas Amazônicas. III. A vegetação lenhosa da campina da Reserva Biólogica INPA-SUFRAMA (Manaus-Caracarai, km 62). Acta Amazônica 5: 225–246.

Anderson, J. A. R. 1963. The flora of the peat swamp forests of Sarawak and Brunei including a catalogue of all recorded species of flowering plants, ferns and fern allies. Gard. Bull. Straits Settlem. 20: 141–228.

Ashton, P. J. 1964. Ecological studies in the mixed dipterocarp forests of Brunei State. Oxford for. Mem. 25.

Beard, J. S. 1946. The Natural Vegetation of Trinidad. Oxford Univ. Press., London.

Beatley, J. C. 1976. Vascular Plants of the Nevada Test Site and Central-Southern Nevada: Ecologic and Geographic Distributions. Energy Research and Development Administration, Washington, D.C.

Bernhard-Reversat, F., C. Huttel & G. Lemee. 1978. Structure and functioning of evergreen rain forest ecosystems of the Ivory Coast. Pp. 557–574 *in* Tropical Forest Ecosystems. UNESCO, Paris.

Bond, P. & P. Goldblatt. 1984. Plants of the Cape Flora, a descriptive catalogue. J. S. African Bot. Suppl. Volume No. 13, National Botanic Gardens, Cape Town.

Boucher, C. 1977. A provisional checklist of flowering plants and ferns in the Cape Hangklip area. J. South African Bot. 43: 57–80.

Brunig, E. F. 1974. Ecological Studies in the Kerangas Forests of Sarawak and Brunei. Borneo Literature Bureau, Kuching, Malaysia.

Bullock, S. H. 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. Biotropica 17: 287–301.

Comstock, J. & J. R. Ehleringer. 1986. Photoperiod and photosynthetic capacity in *Lotus scoparius*. Pl., Cell Envir. 9:609—612.

Croat, T. 1978. Flora of Barro Colorado Island. Stanford Univ. Press, Stanford, California.

Davis, T. A. W. & P. W. Richards. 1934. The vegetation of Moraballi Creek, British Guiana: an ecological study of a limited area of tropical rainforest. Part II. J. Ecol. 22: 106–155.

Eiten, G. 1972. The cerrado vegetation of Brazil. Bot. Rev. 38: 201–341.

Ehleringer, J. & I. Forseth. 1980. Solar tracking by plants. Science 210: 1094–1098.

El-Sharkawy, M. A., J. H. Cock & D. P. Hernández. 1985. Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. Photosyn. Res. 7: 137–149.

Fittkau, E. J. & H. Klinge. 1973. On biomass and trophic structure of the central Amazonian rain forest ecosystem. Biotropica 5: 2–14.

Forseth, I. N. & J. R. Ehleringer. 1983. Ecophysiology of two solar tracking desert winter annuals. III. Gas exchange responses to light, CO_2 and VPD in relation to long-term drought. Oecologia 57: 344–351.

——— & A. H. Teramura. 1986. Kudzu leaf energy budget and calculated transpiration: the influence of leaflet orientation. Ecology 67: 564–571.

Gentry, H. S. 1942. Rio Mayo Plants. Carnegie Inst. Washington Publ. 527, Washington, D.C.

Gibson, A. C. 1983. Anatomy of photosynthetic old stems of nonsucculent dicotyledons from North American deserts. Bot. Gaz. 144: 347–362.

Golley, F. B., J. T. McGinnis, R. G. Clements, G. I. Child & M. J. Duever. 1975. Mineral Cycling in a Tropical Moist Forest Ecosystem. Univ. Georgia Press, Athens, Georgia.

Hartshorn, G. S. 1975. A matrix model of tree population dynamics. Pp. 41–51 *in* F. B. Galley & E. Medina (editors), Tropical Ecological Systems. Springer-Verlag, New York.

Hellmuth, E. O. 1969. Eco-physiological studies on plants in arid and semi-arid regions of Western Australia. II. Field physiology of *Acacia craspedocarpa* F. Muell. J. Ecol. 57: 613–634.

———. 1971. Eco-physiological studies on plants in arid and semi-arid regions of Western Australia. III. Comparative studies on photosynthesis, respiration and water relations of ten arid zone and two semi-arid zone plants under winter and late summer climatic conditions. J. Ecol. 59: 225–259.

Hoover, R. F. 1970. The Vascular Plants of San Luis Obispo County, California. Univ. California Press. Berkeley, California.

Hopper, S. D. & B. R. Maslin. 1978. Phytogeography of *Acacia* in Western Australia. Austral. J. Bot. 26: 63–78.

Howard, R. A. 1968. The ecology of an elfin forest in Puerto Rico. 1. Introduction and composition studies. J. Arnold Arbor. 49: 381–418.

Hulten, E. 1968. Flora of Alaska and Neighboring Territories. Stanford Univ. Press, Stanford, California.

Jessop, J. (editor). 1981. Flora of Central Australia. Reed, Sydney.

Klinge, H., W. A. Rodrigues, E. Brunig & E. J. Fittkau. 1975. Biomass and structure in central Amazonian rain forest. Pp. 115–122 *in* F. B. Galley & E. Medina (editors), Tropical Ecological Systems. Springer-Verlag, New York.

Kruger, F. J. 1979. South African heathlands. Pp. 29–80 *in* R. L. Specht (editor), Heathlands and Related Shrublands, Volume 9A. Elsevier, Amsterdam.

Lathrop, E. W. & R. F. Thorne. 1978. A flora of the Santa Ana Mountains, California. Aliso 9: 197–278.

Lawton, R. M. 1978. The management and regeneration of some Nigerian high forest ecosystems. Pp. 580–588 *in* Tropical Forest Ecosystems. UNESCO, Paris.

Lawton, R. & V. Dryer. 1980. The vegetation of the Monteverde Cloud Forest Reserve. Brenesia 18: 101–116.

Levyns, M. R. 1966. A Guide to the Flora of the Cape Peninsula. Juta, Cape Town.

Lloyd, R. M. & R. S. Mitchell. 1973. A Flora of the White Mountains of California and Nevada. Univ. California Press, Berkeley, California.

Malaisse, F. 1978. The Miombo woodland. Pp. 589–606 *in* Tropical Forest Ecosystems. UNESCO, Paris.

Mooney, H. A. & S. L. Gulmon. 1979. Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. Pp. 316–337 *in* O. T. Solbrig, S. Jain, G. B. Johnson & P. H. Raven (editors), Topics in Plant Population Biology. Columbia Univ. Press, New York.

———, ———, P. W. Rundel & J. Ehleringer. 1980. Further observations on the water relations of *Prosopis tamarugo* of the northern Atacama Desert. Oecologia 44: 177–180.

Nilsen, E. T., M. R. Sharifi & P. W. Rundel. 1984. Comparative water relations of phreatophytes in the Sonoran Desert of California. Ecology 65: 767–778.

———, ———, ———, W. M. Jarrell & R. A. Virginia. 1983. Diurnal and seasonal water relations of the desert phreatophyte *Prosopis glandulosa* (honey mesquite) in the Sonoran Desert of California. Ecology 64: 1381–1393.

Oberbauer, S. F. & B. R. Strain. 1984. Photosynthesis and successional status of Costa Rican rain forest trees. Photo. Res. 5: 227–232.

Ozenda, P. 1958. Flore du Sahara Septentrional et Central. Centre Natl. Rech. Sci., France.

Palgrave, K. C. 1977. Trees of Southern Africa. C. Struik, Cape Town.

Phillips, W. S. 1963. Depth of roots in soil. Ecology 44: 424.

Polunin, O. & B. E. Smythies. 1973. Flowers of Southwest Europe. Field Guide. Oxford Univ. Press, London.

Poore, M. E. D. 1968. Studies in Malaysian rain forest. I. The forest on Triassic sediments in Jengka Forest Reserve. J. Ecol. 56: 143–196.

Prance, G. T., W. A. Rodrigues & M. F. da Silva. 1976. Inventorio florestral de um hectare de mata de terra firme km 30 da Estrada Manaus—Itacoatiara. Acta Amazônica 6: 9–35.

Rundel, P. W., E. T. Nilsen, M. R. Sharifi, R. A. Virginia, W. M. Jarrell, D. H. Kohl & G. B. Shearer. 1982. Seasonal dynamics of nitrogen cycling for *Prosopis* woodland in the Sonoran Desert. Pl. & Soil 67: 343–353.

Sharifi, M. R., E. T. Nilsen & P. W. Rundel. 1982. Biomass and net primary production of *Prosopis glandulosa* (Fabaceae) in the Sonoran Desert of California. Amer. J. Bot. 69: 760–768.

Shearer, G., D. H. Kohl, R. A. Virginia, B. A. Bryan, J. Skeeters, E. T. Nilsen, M. R. Sharifi & P. W. Rundel. 1983. Estimates of N_2 fixation from variations in the natural abundance of ^{15}N in a Sonoran Desert ecosystem. Oecologia 56: 365–373.

Shreve, I. & I. L. Wiggins. 1964. Vegetation and Flora of the Sonoran Desert. Stanford Univ. Press, Stanford, California.

Sugden, A. M. 1982. The vegetation of the Serrania de Macuira, Guajira, Columbia: a contrast of arid lowlands and an isolated cloud forest. J. Amer. Arbor. 63: 1–30.

Sylvester-Bradley, R., L. A. de Oliveira, J. A. de Podesta Filho & T. V. St. John. 1980. Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing *Azospirillum* spp. in representative soils of central Amazonia. Agro-ecosystems 6: 249–266.

Szarek, S. R. & R. M. Woodhouse. 1978. Ecophysiological studies of Sonoran Desert plants. IV. Seasonal photosynthetic capacities of *Acacia greggii* and *Cercidium microphyllum*. Oecologia 37: 321–329.

Thomas, J. H. 1961. Flora of the Santa Cruz Mountains of California. Stanford Univ. Press, Stanford, California.

Ullmann, I. 1985. Tagesgänge von Transpiration und stomätarer Leitfähigkeit sahelischer und saharischer Akazien in der Trockenzeit. Flora 176: 383–409.

Walter, H. 1973. Vegetation of the Earth and Ecological Systems of the Geo-biosphere. 2nd edition. Springer-Verlag, Berlin.

Whibley, D. J. E. 1980. Acacias of South Australia. D. J. Woolman, Adelaide.

Zabriskie, J. G. 1979. Plants of Deep Canyon. Philip L. Boyd Deep Canyon Research Center, Riverside, California.

LEGUME FRUITS—THE STRUCTURE:FUNCTION EQUATION

J. S. Pate[1]

Abstract

The principal function of the legume fruit is to attract, temporarily store, process, and mobilize to its seeds a range of essential organic and inorganic solutes derived from the parent plant. This it performs with high efficiency in terms of consumption of water, conservation of carbon and nutrient mobilization from pod to seeds. A number of structural specializations interact in attaining these functions. Low stomatal frequency and heavy cutinization of the outer epidermis promote good water use efficiency but restrict CO_2 fixation from the external atmosphere and transpiration-linked attraction of predominantly xylem-borne minerals such as Ca and Mn. With poor ventilation CO_2 accumulates to high concentrations (0.3–2.5% v/v) in the fruit gas space, leading to effective reutilization of respired carbon during the day by chlorenchyma of the mesocarp and, in some species, also by a chloroplast-containing inner epidermis. The xylem network of the fruit exhibits the potential for fully effective exchange of water between seeds, pod wall, and parent plant. Diurnally-reversing xylem flow occurs in some species, net intake by the fruit at night attracting xylem-mobile nutrients, back flow to the plant in the day ridding the fruit of excess water entering by mass flow in the phloem. The lateral vasculature of the pericarp varies in architecture between species. The thick-walled fruits of certain species possess inward projecting veinlets, while in others a mycelium-like network of internal phloem is present. Either system might assist in nourishing developing endocarp fibers or in filling and emptying the pericarp's reservoir of stored solutes. The special case of subterranean achlorophyllous fruits is considered. Case studies are presented of the relative efficiencies with which selected grain legume species convert imported solutes into storage products of seeds.

I. Introduction

The fruits of Leguminosae vary greatly in external form, ranging from the legume proper, typical of most members, to lomentum- or samara-like structures in certain genera (see Augspurger, this volume). Within each of these categories there also exists wide variation in readily discernible features such as general shape, length, and diameter of the fruit, and the extent of lateral flattening of the pericarp wall. Both aerial and geocarpic fruits are encountered in the family. Some species possess an extensive internal gas cavity, others do not. Some have an extremely thin pericarp, others a very thick and fleshy one. The distribution of chlorophyll-containing tissues within fruits is highly variable. Most fruits develop a highly fibrous pericarp, but some, especially domesticated cultivars of grain legumes, may totally lack or be notably deficient in pod wall fibers. Finally, within the family as a whole, enormous variation exists between species in seed number and size, in respect of times taken for fruit and seed to reach maturity, and in the allometric relationships between pod and seed weights and volumes during the ripening period.

Faced with this bewildering array of variation and with extremely fragmentary information on the anatomical, physiological, and biochemical characteristics of fruits of all but a few legume genera, it becomes virtually impossible to formulate a generalized structure:function equation for legume fruits as a whole. What can be accomplished, however, is to define and examine in turn the structural qualities displayed generally or restrictedly by those members of Leguminosae which have been studied, and to discuss how these features relate singly or collectively to fruit functioning. Special emphasis will be placed on the productiveness of the fruit in terms of utilization of resources

[1]Botany Department, University of Western Australia, Nedlands, Western Australia 6009, Australia.

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of water, carbon, nitrogen, and other essential elements. Photosynthetic contributions, if any, to fruit carbon balance will be considered, and assessments made of the efficiency with which reserves stored temporarily in the pericarp are channeled into maturing seeds.

For the most part, attention will be focused on cultivated grain legumes (notably of the genera *Pisum, Vigna,* and *Lupinus*) with which the author is reasonably familiar and for which both structural and functional studies are reasonably well documented. The paper by Van Staden et al. in this volume is devoted to structure:function relationships of seeds, so this paper will concentrate particularly on the pericarp of the fruit and its role in nourishment of developing seeds.

II. Structural Features of the Fruit Pericarp and Their Physiological Significance

As shown in a contribution (Pate & Kuo, 1981) to an earlier symposium on the biology of Leguminosae, the pod wall of legumes displays a number of highly variable features, notably in relation to the number of cell layers and tissue types represented within its mesocarp and endocarp, its vascularization, and the nature of its outer and inner epidermal layers.

Especially important to the water economy of aerially borne fruits are the surface area:volume relationships of the developing pod, the extent of its tissue reserves of water, and specialized features of its outer epidermis such as low stomatal frequency, partial obstruction of stomata by cuticular flanges, and heavy cutinization. As evident from a number of studies on domesticated species, legume fruits exhibit extremely high water use efficiency and record correspondingly low transpiration ratios during growth. There is a considerable element of variation in this respect between species. The fast-maturing, large-seeded, cylindrical fruits of cowpea (cv. Vita 3), for example, exhibit a lesser transpiration ratio (8 mls H_2O transpired/g fruit dry matter gain; Peoples et al., 1985) than the slower developing fruits of white lupine (23 ml/g; Pate et al., 1977) and soybean (20 ml/g; Layzell & LaRue, 1982), and the dorsiventrally flattened fruits of pea (20 ml/g; Flinn et al., 1977).

Pod wall thickness may vary from only a few millimeters in the inflated fruits of genera such as *Genista monosperma* and *Gompholobium* spp., to up to 1–2 cm in the fleshy edible pericarp of genera such as *Phaseolus, Vigna, Prosopis,* and *Ceratonia,* or to 2–4 cm thick in *Vatairea guianiensis* (C. H. Stirton, pers. comm.). At the other extreme one finds the paper thin walls of *Psoralea* spp., where a large fleshy calyx may act as an important nutrient reserve for seed development (C. H. Stirton, pers. comm.). If it is generally true that nutrient reserves of pods increase with increasing volume of storage parenchyma in the pod, thick fleshy fruits with a low seed:pod mass ratio should be able to furnish a greater proportion of the nutrient requirements of their developing seeds than thin-walled, large-seeded fruits. However, certain thick-walled fruits are distinctly woody (e.g., *Castanospermum, Ceratonia*), and nutrient storage may well be reduced proportionally to the amounts of sclerenchyma present. Data are simply not available to test this hypothesis fully, but the information provided for two grain legumes in Table 1 indicates that considerable differences may exist between species in this respect, as well as in the efficiency with which the senescing pod is capable of mobilizing various macronutrients and micronutrients to its seeds. The possible role of the pericarp in buffering seed development against physiological or environmental stress is particularly well discussed by Muntz et al. (1978).

Virtually all aerially borne legume fruits are green due to chlorenchyma in the outer mesocarp of the pod wall. Some species (e.g., pea [*Pisum sativum*], lupine [*Lupinus albus* L.], lentil [*Lens culinaris*], and chickpea [*Cicer arietinum*]) also possess a chloroplast-containing inner epidermis lining the gas cavity of the fruit (Flinn et al., 1977; Pate & Kuo, 1981). As shown for the 'Greenfeast' cultivar of pea by Atkins et al. (1977), distribution of chlorophyll in the pod accurately reflects distribution of the CO_2-fixing enzymes ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase) between pod mesocarp and

TABLE 1. Mobilization of a range of mineral reserves from pods of two grain legumes and the significance of such mobilization to the nutrient requirements of the seeds. Data from Hocking & Pate (1977).

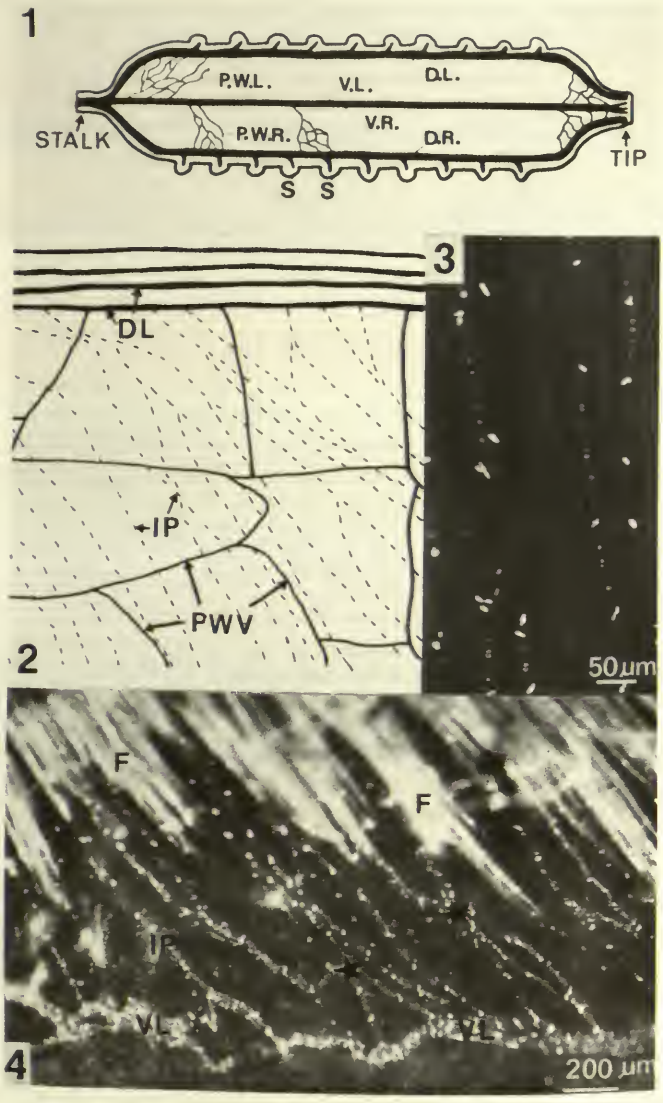
	Efficiency of mobilization of mineral reserves of pod to seed during fruit maturation* %		Loss from pod as a proportion of amount finally accumulated by seeds of fruit* %	
	Lupinus albus (cv. Neutra)	*Pisum sativum* (cv. Greenfeast)	*Lupinus albus* (cv. Neutra)	*Pisum sativum* (cv. Greenfeast)
Macronutrients				
Nitrogen	83	88	23	15
Phosphorus	73	82	23	13
Potassium	65	48	31	14
Magnesium	47	54	39	14
Calcium	8	9	7	12
Micronutrients				
Iron	33	32	18	9
Zinc	54	37	20	7
Manganese	41	27	31	9
Copper	35	32	12	5

*Each value refers to the proportional loss of an element from the time when it had achieved maximum content in the pod to the time when the pod was fully dried out and the seeds fully ripe.

endocarp, there being roughly three times more CO_2-fixing capacity extractable from the 'Greenfeast' pea pod's outer photosynthetic layers than the inner ones. Functioning of these tissues in photoassimilation of carbon dioxide has been demonstrated by enclosing whole illuminated pea fruits in an atmosphere labeled with $^{14}CO_2$, whereupon their outer mesocarp layers are labeled mostly with ^{14}C sucrose. Later, this labeled sugar can be traced to seeds. Alternatively, if $^{14}CO_2$ is injected into the gas cavity of an illuminated fruit, the photosynthetic inner epidermis is labeled, and the resulting ^{14}C assimilates are also eventually transfered to the seeds (Pate & Flinn, 1973; Atkins et al., 1977; Pate, 1975, 1985).

As a direct consequence of its poor ventilation, legume fruits typically accumulate high levels of CO_2 in their gas spaces, e.g., 0.3–2.2% (v/v) in cowpea (M. B. Peoples, unpublished data), 0.5–1.5% in white lupine (J. S. Pate, unpublished data), and 0.05–2.5% in garden pea (Flinn et al., 1977). Highest gas space concentrations of CO_2 are evident at night, despite cooler night temperatures leading to lesser rates of CO_2 being respired from seeds than during the day. Internal CO_2 levels in the gas space are reduced by pod photosynthesis during the day, and artificial darkening of a fruit during the day can lead to even higher CO_2 levels in the pod gas space than are observed at night (Flinn et al., 1977). Despite the high level of PEPCase in pod and seed tissues, and the fleshy nature of the pods of many legume species, there is no evidence of significant dark fixation of CO_2 by Crassulacean Acid Metabolism. However, the high levels of CO_2 in pod tissues may have particular significance to effective photosynthetic functioning of PEPCase (see Atkins et al., 1977; Pate, 1984) and to reducing the respiration rate of seeds (Atkins & Flinn, 1978).

As described initially by Woodcock (1934) for french bean (*Phaseolus vulgaris*), and more generally for other species by Fahn & Zohary (1955), Esau (1965), Pate et al. (1974), and Pate & Kuo (1981), the vasculature of the legume fruit comprises sets of longitudinal strands that run the length of the upper dorsal (placental) and lower ventral (nonplacental) sutures of the fruit and end blindly at the distal tip of the fruit (Fig. 1). The dorsal strands (DL, DR) serve the seeds, and a network of lateral strands (PWL, PWR) ramifying through the mesocarp of the side walls of the pod cross-connect the ventral and dorsal longitudinal strands at many points throughout the length of the pod.



FIGURES 1–4.—1. General vasculature of a typical legume fruit showing longitudinal vascular components of left- and right-hand sides of venter (VL, VR), dorsum (DL, DR), and network of veins (only partly shown) in lateral walls of pericarp (PWL, PWR). S represents seeds attached to left and right margins of dorsum. Note blindly ending strands in fruit tip (not to scale). 2–4. Unusual mycelium-like network of internal phloem in inner mesocarp of lateral pod wall of cowpea *Vigna unguiculata* (L.) Walp cv. Vita 3 (see Kuo & Pate, 1985).—2. Conformation of internal phloem (IP) in relation to overlying vascular strands in the pod wall (PWV) and longitudinal strands of dorsum (DL) (not to scale).—3. High-power view of surface of inner mesocarp (fresh material) stained with aniline blue and viewed by fluorescent microscopy to show fluorescence of callose of single files of sieve elements ramifying through the tissue immediately above the endocarp fibers. The latter fluoresce slightly (faint parallel lines on photomicrograph).—4. Similarly treated material as Figure 3, showing general orientation and branching (stars) of network of internal phloem (IP) and the joining of this network onto the phloem of the longitudinal vasculature (VL) of the ventral suture of the pod, beyond the sheet of endocarp fibers (F).

Our earlier study (Pate & Kuo, 1981) described species specific differences in venation of pod walls. Some species (e.g., *Phaseolus vulgaris, Psophocarpus tetragonolobus, Vigna unguiculata,* and *Lens culinaris*) were recorded as having a closed network with no free-ending veinlets, others an open network with veinlets either in the same plane as the parent network (*Vicia faba, Cicer arietinum*) or projecting inward toward the endocarp (*Lupinus albus, Pisum sativum*). In *L. albus* the free veinlets consist only of phloem elements; in *P. sativum* they consist of both xylem and phloem.

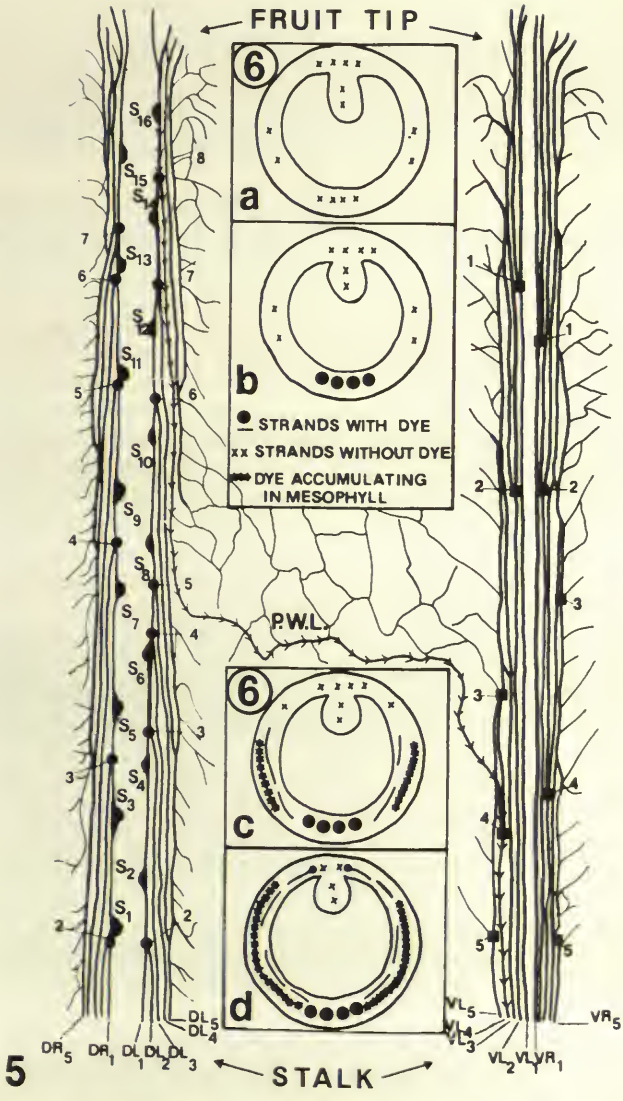
Recently (Kuo & Pate, 1985) a highly unusual mycelium-like network of internal phloem has been described in the inner mesocarp of certain genotypes of cowpea *(Vigna unguiculata)* (see Figs. 2–4). This additional vascular system does not relate conformationally to or connect at any point with the above conventional (xylem and phloem) closed venation of the outer mesocarp (Fig. 2). The internal phloem consists of a finely branched reticulum of mostly single sieve elements and associated phloem parenchyma, whose orientation is mainly parallel with that of the closely adjacent endocarp fibers (Fig. 3). However, this network of internal phloem extends beyond the fiber sheet to join with phloem of the dorsal and longitudinal vascular strands of the sutures of the fruit (Fig. 4). Curiously, internal phloem of this apparently unique nature has been observed so far only in *V. unguiculata* and, even more interestingly, only in certain genotypes of this species—not necessarily those possessing endocarp fibers, large seeds, or especially thick pod walls. Kuo & Pate (1985) speculate that this unique type of phloem network might function in providing assimilates for fiber development in the adjacent endocarp, or in the filling and draining of solute reservoirs in adjacent fleshy layers of the pod wall. It may thus be considered analogous in function with the downward-projecting veinlets mentioned above for other legume species.

A full appreciation of the complexity of the whole vasculature of the cowpea fruit demands a thorough examination of all parts of its architecture, employing both cleared preparations of whole fruits and high resolution light microscopy of sections through a number of strategic locations in a fruit. Such information has been recently presented for the 'Vita 3' cowpea (Pate et al., 1985), one of the genotypes mentioned above as possessing a network of internal phloem.

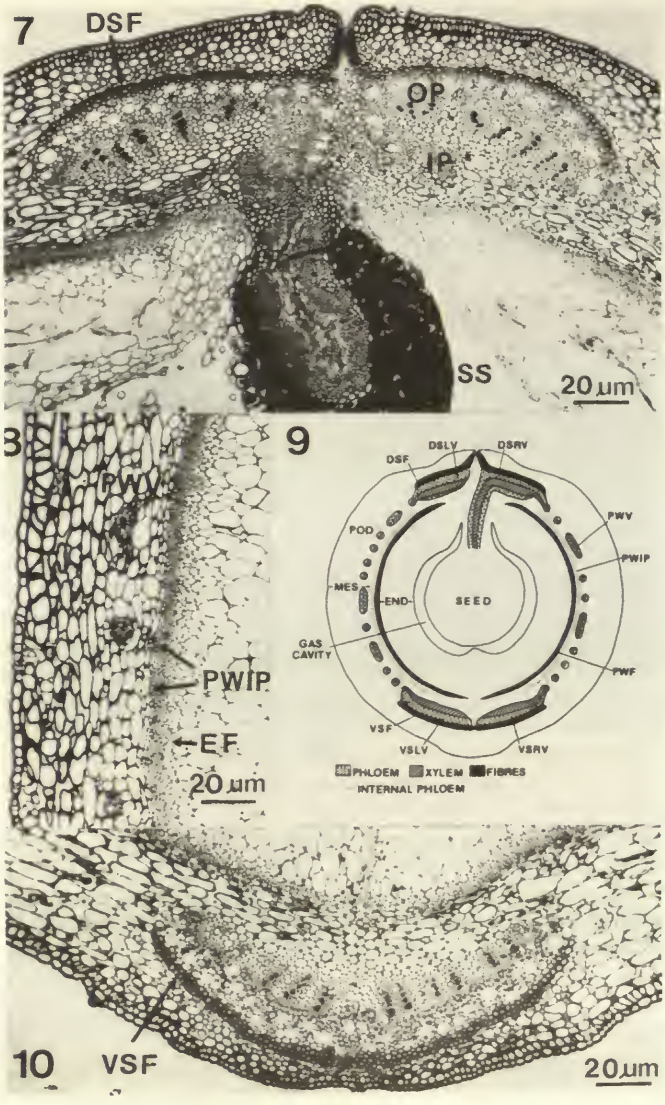
The xylem of the cowpea fruit comprises a completely integrated network (Fig. 5). Distinct longitudinal strands of xylem elements, originating from vascular tissues of the fruit stalk, run along the left and right halves of both the ventral (lower nonplacental) ($VL_{1\text{-}5}$ and $VR_{1\text{-}5}$, Fig. 5) and dorsal (upper placental) ($DL_{1\text{-}5}$ and $DR_{1\text{-}5}$, Fig. 5) of the fruit. The files of xylem strands in the venter bifurcate as they pass up the fruit (see numbered branching points VL and VR, Fig. 5), and after running for a short distance together, the outermost of each branch pair moves out into the lateral vein network. Branching more or less compensates for lateral losses of daughter strands so that 8–10 groups of xylem elements are regularly visible in transections of any region of the ventral suture of the pod except close to its tip (Figs. 5, 10). The dorsal (placental) longitudinal network, in contrast, shows successive fusions between its members (numbered DR_1 and DL_1, Fig. 5), with innermost members of the left and right halves of the dorsum respectively serving seeds (S_1–S_{16}, Fig. 5) attached alternately to left- or right-hand margins of the pod (Fig. 2). At the same time, strands of the lateral pod network merge progressively into the dorsal vasculature, thus replacing longitudinal strands lost through fusions and vascularization of seeds.

Serial sectioning of seed stalks (Figs. 7, 9) shows continuous xylem strands running from the dorsum to the seed coat. As shown in Figure 5, all seeds connect directly with the fruit stalk via the longitudinal vasculature of the dorsum, but each seed also has a specific vascular association with adjacent regions of the lateral vein network, and thence across the pod wall to specific longitudinal elements of the ventral vasculature. An example of such a connection is traced by arrows in Figure 5.

Transverse sections of the pod wall, dorsum, venter and seed stalk of the 'Vita 3' cowpea fruit (Figs. 7–10) provide evidence that normal outer phloem accompanies xylem throughout all major parts of the vasculature (PWV, DSLV, DSRV, VSLV, and VSRV; Figs. 7–10). Additionally, internal strands consisting only of phloem run along the inner face of the dorsum and into the seeds



FIGURES 5, 6.—5. Organization of vascular strands in the dorsum, venter, and part of the left half lateral wall of a fruit of 'Vita 3' cowpea. Longitudinal strands of dorsum (DR, DL) and venter (VR, VL) are numbered as right- and left-hand series respectively, and fusions or branching of the strands are indicated by numbered circles (fusions) or squares (branches) along the length of the strands. Positions of seeds are shown (S_1–S_{16}), and a cross connection from the dorsal to the ventral vasculature via the pod wall network (PWL) is indicated by arrows (drawn to greatly foreshortened vertical scale, but conformation of vasculature accurately represented, as seen from a cleared specimen of a whole fruit).—6. Results of transpirational feeding of the apoplast marker dye acid fuchsin through the cut shoot of fruiting plants of cowpea. Distribution of dye in transverse sections of fruits is shown after one hour of feeding: (a) Dye fed in mid afternoon, no dye enters the fruit through xylem. (b) Dye fed in early morning, dye enters only along ventral strand. (c) Dye fed early evening, dye enters xylem of ventral strand and lower lateral veins and accumulates in mesophyll at sites of transpiration. (d) Dye fed in late night, dye present in quantity in all parts of pod vasculature and mesophyll but not in seed stalks or seeds (redrawn with modifications from Pate et al., 1985; see text for further description of daily xylem exchanges of fruit).



FIGURES 7–10. Vasculature of transection of mid region of fruit of cowpea (*Vigna unguiculata* (L.) Walp. cv. Vita 3).—7. Dorsal regions of fruit with seed stalk (SS) connected to left-hand vasculature of dorsal suture. Fiber cap to vascular strand labeled DSF, xylem elements of strands are marked in black to ease recognition in the micrograph, OP outer phloem, IP inner phloem.—8. Lateral wall of pod showing pod wall vein network (PWV) and internal phloem (PWIP) just outside developing endocarp fibers (EF).—9. Line diagram of vasculature of whole transection, showing internal and external phloem and continuity of vascular connection to seed, comprising xylem and derivatives of inner and outer phloem. MES = mesocarp; END = endocarp; other labeling as described in Figures 7, 8, 10.—10. Ventral region of fruit showing vasculature of left- and right-hand halves of ventral suture, capped by a continuous band of fibers (VSF). Xylem vessels of ventral strands in the micrograph are blackened to aid in recognition.

(IP, Figs. 7, 9). This phloem component also connects laterally with the network of internal phloem (PWIP, Figs. 8, 9) in the inner mesocarp of the fruit.

The highly integrated nature of the fruit's total vasculature has two important physiological implications. First, the potential exists for xylem-borne water to exchange freely and rapidly between all parts of the pod and seeds as well as between the whole fruit and parent plant. This is an essential prerequisite of the concept of reversible xylem flow of water between fruit and plant expounded in a later section of this chapter. Second, since each seed is capable of drawing directly or indirectly upon assimilates entering through the dorsal and ventral vasculature as well as upon those emanating from pod walls, damage to specific parts of a fruit's vasculature will be likely to have only minimal effects on seed nutrition. This is vividly demonstrated in cowpea fruits by experiments (J. S. Pate, J. Kuo & M. B. Peoples, unpublished data) in which dorsal or ventral vascular strands were severed early in fruit development and cut again frequently thereafter to prevent reestablishing of vascular connections by callus formation. Growth of the fruit and filling of its seeds then proceeded in an apparently normal fashion, despite only one or other half of the fruit's longitudinal vasculature being available for intake of assimilates and water. The same result was obtained if whole sections of lateral wall were removed from a developing fruit, seeds developing quite normally even in positions only a centimeter or so away from where pod wall tissue had been completely excised.

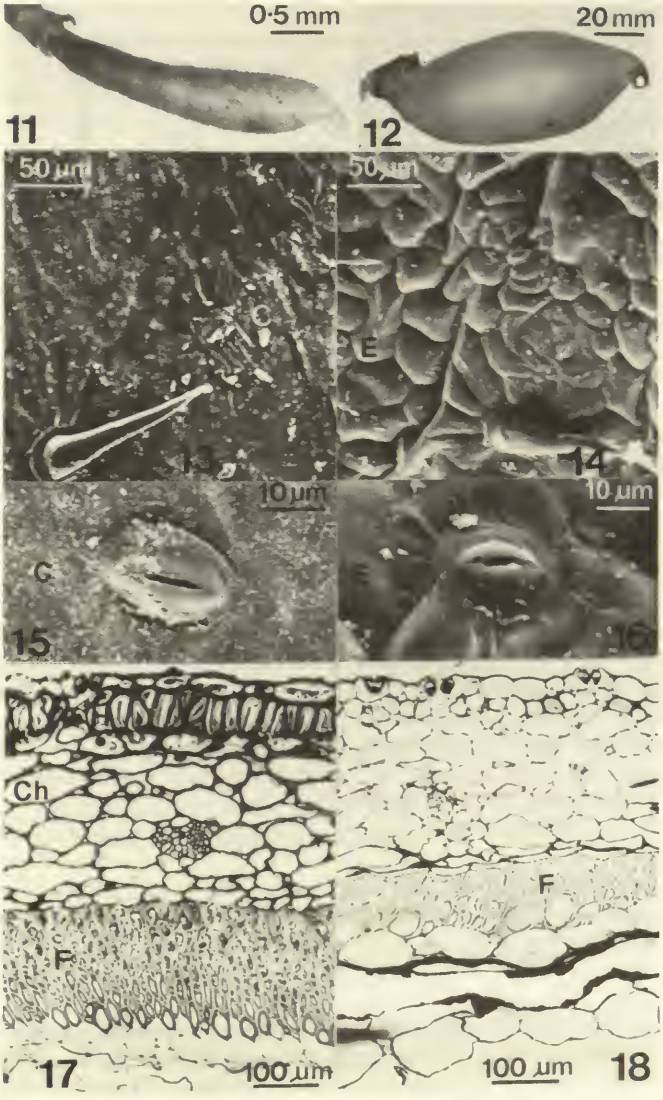
The fibrous layers of the endocarp of the fruit are of course essential to the explosive mechanism of dehiscence typical of virtually all aerial, legume-type carpels of Leguminosae (see Van Staden et al., this volume). In selecting for nonshattering pods, and in some species for edible whole fruits, man has produced cultivars showing reduced or even total absence of wall fibers (see Pate & Kuo, 1981; Pate, 1985). As the fiber layers of progenitor species may represent a significant irretrievable investment of carbonaceous material within the fruit, it is reasonable to assume that domesticates with lesser fiber content should be commensurately more effective in channeling imported photosynthate into their seeds. Additionally, since a thickly fibrous endocarp will greatly attenuate light penetration to the pod interior, cultivars of species possessing an inner photosynthetic epidermis would be more likely to utilize this system efficiently if such fibrous layers were reduced or absent.

Finally, attention should be drawn to the anatomical characteristics of fruits borne underground, as encountered, for example, in certain species of *Voandzeia, Arachis, Trifolium, Glycine,* and *Vigna.* In certain tropical Australian species of the last two genera, plants carry both green aerial dehiscent fruits and achlorophyllous underground nondehiscent fruits. The latter develop from cleistogamous flowers borne on fully subterranean rhizomes. In one unnamed species of *Vigna* (Figs. 11–18) the aerial fruits (Fig. 11) are of cylindrical shape and are 3–5 seeded, in contrast with the spheroid, single or double seeded underground fruits (Fig. 12). Pod walls of the underground fruits totally lack chlorophyll and hypodermal fibers (Fig. 18), have reduced fiber layers in their endocarp (Fig. 18), and possess a thin-walled, poorly cutinized hydrophilic epidermis (Figs. 14, 16); the aerial fruits show contrasting structural features (Figs. 13, 15, 17). The significance of a hydrophilic outer surface to fruits of geocarpic species is considered later in relation to nutrient uptake from the surrounding soil.

Virtually no attention has been given to the anatomy and gas exchange characteristics of the various types of wind-dispersed legume fruits described by Augspurger elsewhere in this volume.

III. The Carbon Balance of Grain Legume Fruits

Quantitative inventories of carbon utilization by developing fruits have been constructed for cultivars of both cowpea and garden pea. The experimental procedure is to assemble information on accumulation of carbon in pod and seed dry matter, day:night exchanges of CO_2 by the whole fruit, and CO_2 losses to the pod gas space day and night by the respiring seeds (see Pate, 1984). From



FIGURES 11–18. Fruit morphology and pod wall anatomy of green cylindrical 3–5-seeded aboveground fruits (Figs. 11, 13, 15, 17) and spheroid achlorophyllous 1- or 2-seeded underground fruits (Figs. 12, 14, 16, 18) of an unnamed Northwest Australian species of *Vigna*. Single plants bear both types of fruits simultaneously. Note contrasting scanning electron microscope views of outer epidermis, the aerial fruit with a thick waxy cuticle (C, Figs. 13, 15), the geocarpic fruit with a thin hydrophilic cuticle and convex surfaces to its epidermal cells (E, Figs. 14, 16). Both fruits carry trichomes (Figs. 13, 14) and stomata (Figs. 15, 16) with pronounced ridges on the inner faces of their guard cells. Pod wall anatomy of the aerial fruit shows a hypodermis (H) and chlorenchyma (Ch) in the outer mesocarp (Fig. 17), features not present in the underground fruit (Fig. 18). Note also much more fibrous endocarp of the dehiscent aerial fruit (F, Fig. 17) than the nondehiscent subterranean fruit (F, Fig. 18).

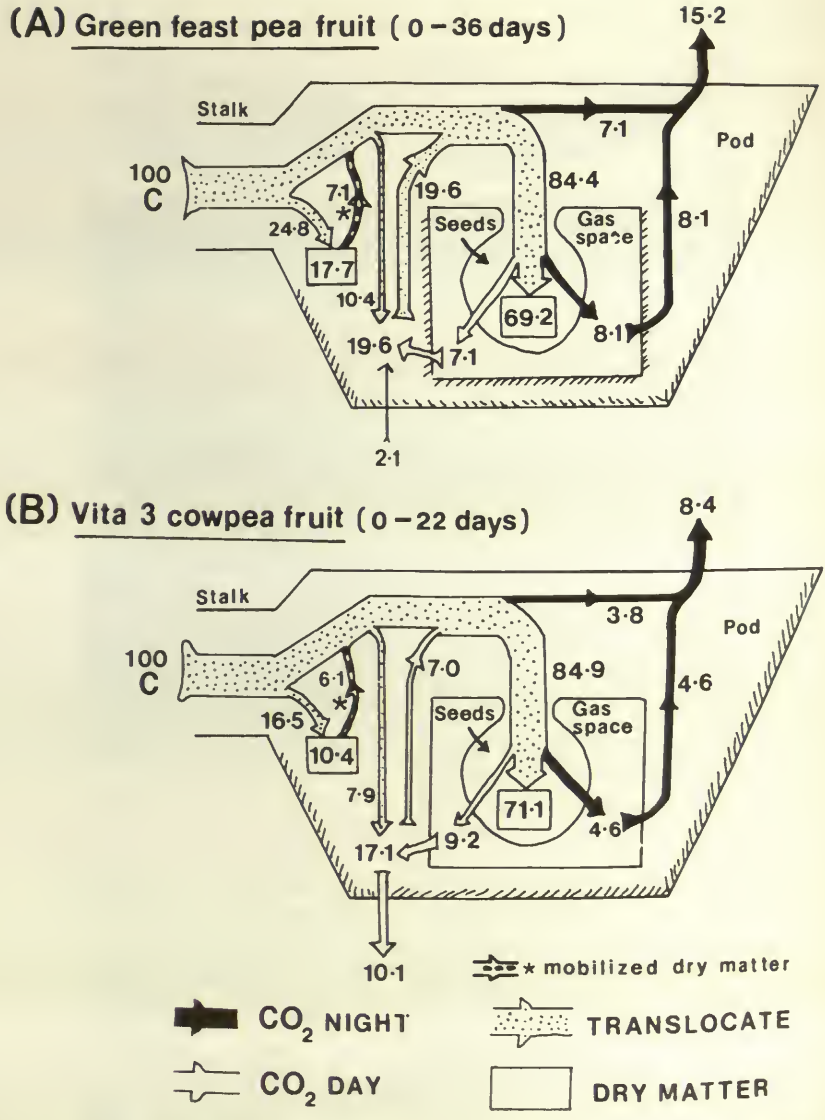


FIGURE 19. Carbon economy for the whole ripening period of fruits of two species of grain legumes. Each budget for carbon depicts the fate of 100 units by weight of carbon entering through the seed coat. Note the poorer CO_2 economy of the cowpea cultivar, but its lower investment of carbon in nonmobilizable structural proteins in its pod (redrawn with modifications from Pate, 1984).

this primary information, one can then determine what contribution the pod wall makes to refixation of the fruit's respired carbon and what proportion of the fruit's net intake of carbon is successfully incorporated into dry matter of the seeds. The relevant profiles for carbon flow for the two test species are shown in Figure 19, each flow chart expressed on the basis of a net intake of 100 units by weight of carbon from the parent plant. The two fruits showed virtually identical efficiencies of conversion (69–71%) of imported carbon into dry matter of seeds. However, the 'Greenfeast' cultivar pea fruit (Fig. 19A) recorded a net (day and night) loss of only 13.1 units by weight of

carbon as CO_2 to the external atmosphere, compared with 18.5 in the 'Vita 3' cowpea fruit (Fig. 19B). The total conservation of CO_2 by the pea pod was equivalent to 19.6 units of carbon, consisting of 2.1 units net gain from the atmosphere during photoperiods of the early life of the fruit, 7.1 units recovered as seed respired CO_2 via the pod gas cavity, and 10.4 units as the pod's own respiration. For 'Vita 3' cowpea the corresponding advantage was only 7 units of carbon, due to only partial recovery of the respired CO_2 of pod and seeds during the day. Nevertheless, the 'Vita 3' cowpea fruit made a relatively small initial investment of carbon in pod structure (16.5 units of carbon) in comparison with the 'Greenfeast' pea (24.8 units); and since almost as great an amount of carbon was retrieved from this pool to the benefit of seeds of cowpea (6.1 units) than of pea (7.1 units), correspondingly lesser amounts of carbon were left behind in the dried pod wall of cowpea (10.4 units) than in pea (17.7 units). This more efficient mobilization of pod reserves more than compensated for the poorer performance of the cowpea fruit in terms of photosynthetic fixation of carbon.

IV. The Water Balance of Legume Fruits

A quantitatively based modeling technique relating the exchanges of carbon, nitrogen, and water that take place between fruit and parent plant was first devised by Pate et al. (1977) for study of the economy of white lupine fruits, and was subsequently adapted for soybean (Layzell & LaRue, 1982) and cowpea (Peoples et al., 1985). Construction of each empirically based model requires knowledge of the amounts of carbon and nitrogen incorporated into fruit dry matter during specific intervals of growth, the net exchanges of carbon with the fruit's external atmosphere, and the C:N weight ratios and concentrations of carbon and nitrogen present in the solute streams entering the fruit through xylem and phloem. The latter are assessed by collection and analysis of fruit stalk tracheal (xylem) sap or fruit phloem bleeding sap respectively (see Pate et al., 1974 [lupine]; and Pate et al., 1984 [cowpea]). As initially constructed, each model assumes unidirectional inputs of carbon and nitrogen by mass flow via xylem and phloem, and is based on determining which mixture of xylem and phloem streams meets precisely the recorded carbon and nitrogen utilization by the fruit during a specific period of its growth. The validity of the model is then tested on the basis of whether this mixed stream matches precisely the recorded consumption of water by the fruit, as assessed by transpiration loss and net loss or gain in fruit tissue water over the experimental period.

Applied to the whole maturation period of the fruit of white lupine (Pate et al., 1977; Pate, 1984), xylem is estimated to supply 60% of the fruit's water, phloem virtually all (98%) of the carbon and the bulk (89%) of the nitrogen. With this 60:40 (v/v) mixture of xylem and phloem streams, an amount of water is predicted to enter by mass flow, satisfying over 95% of the fruit's requirement for water.

When applied to cowpea, however, a gross misfit becomes apparent within the budget data; namely, that in order to meet the nitrogen intake of the fruit, much greater amounts of water would have to enter through vascular channels than are ever accounted for by tissue water changes or fruit transpiration (Peoples et al., 1985). This discrepancy is best resolved by assuming that excess water entering the fruit by mass flow in xylem and phloem returns in some manner to the parent plant. The model for cowpea (see Peoples et al., 1985) shown in Figure 20 lists this component as "recycled" water and rates it equivalent to 23% of the fruit's gross intake of water during the first half (Fig. 20A) and 70% during the second half (Fig. 20B) of fruit development. This is equivalent in absolute terms to a return from fruit to plant of 0.5–3 ml water per day during all but the very early stages of fruit development (see Summerfield et al., 1985).

Further insight into the water relationships of the cowpea fruit comes from considering its water balance on a daily basis (see Pate et al., 1985; Atkins et al., 1986). Study has to be made of daily profiles in transpiration and day:night CO_2 exchanges of the fruit. Mass (200 fruit) harvests of similarly aged and sized fruits are also made at dawn and dusk of successive days of a study period

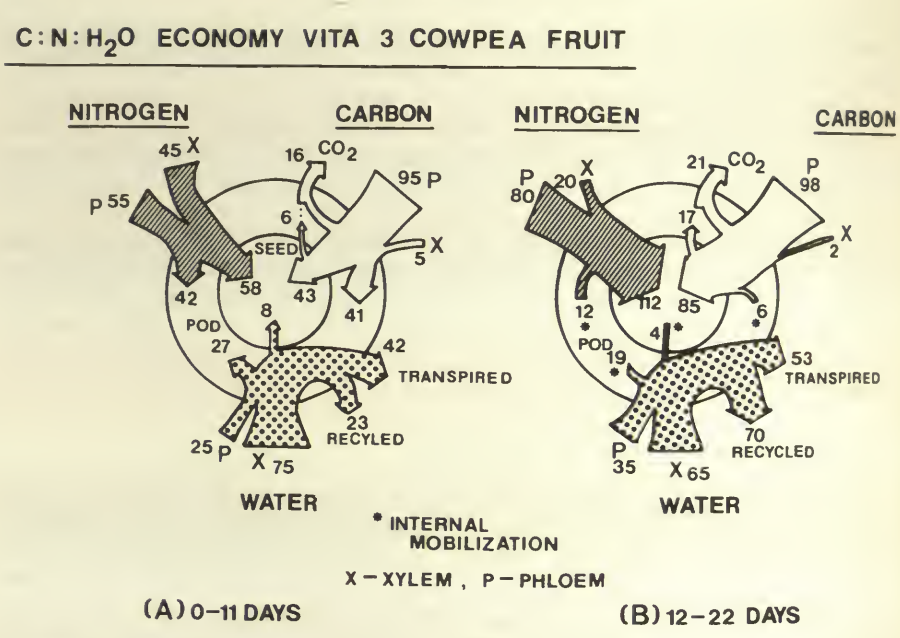


FIGURE 20. Proportional intake of carbon, nitrogen, and water through xylem (X) and phloem (P) during the two consecutive halves of the ripening period of a 'Vita 3' cowpea (*Vigna unguiculata*) fruit. Components of the fruit's budget are expressed relative to a net intake of 100 units of C, N, or H_2O. The recycled component of the water budgets is assumed to be implemented by diurnally reversing xylem flow of water, as suggested in the text. Ratios of absolute amounts by weight of C, N, and H_2O consumed by the fruits are C:N:H_2O = 16:1:546 for 0–11 days, 13:1:130 for 12–22 days. Data from Peoples et al., 1985.

to assess daytime and nighttime changes in fruit fresh weight and gains of carbon in dry matter. By comparing fresh and dry weight data, it can then be ascertained whether day:night changes are occurring in fruit tissue water. Then, assuming that carbon enters by mass flow through phloem at the concentration of this element recorded for phloem sap, the amount of water entering the fruit by phloem to meet the recorded day and night consumption of carbon by the fruit can be computed. When these amounts are matched against the recorded transpiration loss and tissue water change in the fruit, a final assessment can then be made of whether extra water has been imported by the fruit through xylem, or whether the fruit is indeed showing a current excess of water and is therefore likely to be exporting this surplus back to the plant through the xylem.

Following this protocol, data have been collected for the 'Vita 3' cowpea fruit during two specific intervals of its 22-day maturation program (Table 2). The fruit is found to transpire 2–4 times faster in the day than at night and to import carbon through phloem at rates showing comparable or slightly greater diurnal amplitudes. However, the fruit shrinks appreciably and records net losses of tissue water during the day at times of high foliar transpiration, but recovers this deficit in tissue water at night once foliar transpiration ceases and xylem water potentials become less negative. Accordingly, in the examples shown in Table 2, a net influx of water through xylem is predicted to occur at night, a reverse xylem flow of excess water back to the parent plant during the day.

The concept of reversing xylem flow between source and sink organs is by no means new, being embodied in the original exposition of the mass flow theory of phloem transport by Münch (1930) and later alluded to and tested experimentally in relation to fruits of a range of species, including legumes (e.g., the study of Mix & Marschner [1976] on *Phaseolus*) and nonlegumes (e.g., the studies of Clements [1940] on sausage tree [*Kigelia africana* (Bignoniaceae)] and of Ziegler [1963]

TABLE 2. Day:night water balance of the cowpea (*Vigna unguiculata* (L.) Walp cv. Vita 3) fruit at two stages during the second half of its development.

Item of fruit's carbon or water balance	15–16 days after anthesis[1]		17–18 days after anthesis[2]	
(mg or ml fruit)	Night	Day	Night	Day
1. Intake of C by fruit (mg C)[3]	39.0	139.1	41.2	152.3
2. Intake of H_2O in phloem (ml)[4]	0.46	1.65	0.49	1.81
3. Change in tissue H_2O of fruit (ml)	+0.55	−0.44	+0.43	−0.63
4. Transpiration loss of H_2O (ml)	0.38	1.37	0.51	1.51
5. H_2O required to balance 3 + 4 (ml)	0.93	0.93	+0.94	−0.88
6. Net flux of H_2O in xylem (5−2) (ml)	+0.47 (in)	−0.72 (out)	+0.45 (in)	−0.93 (out)

[1]Data from Atkins, Pate & Peoples (1986).
[2]J. S. Pate, M. B. Peoples & C. A. Atkins (unpublished data).
[3]Comprises increment in dry matter C plus net loss (day and night) of C as CO_2 to the atmosphere.
[4]Assumes mass flow in phloem and 84 mg C/ml^{-1} phloem sap.

on *Cucurbita* [Cucurbitaceae] and other species). The ^{45}Ca feeding studies of Mix & Marschner (1976) on *Phaseolus vulgaris* are particularly relevant to testing of the concept, as are our own recent studies on cowpea (Pate et al., 1985) in which distribution of apoplast-mobile dyes (acid fuchsin, ^{3}H-inulin) and time course studies of $^{3}H_2O$ and $^{32}PO_4$ labeling of fruits were examined following administration of these tracers to root or leaf through xylem or phloem. These studies (e.g., the acid fuchsin feeding experiment summarized in Fig. 6) demonstrate a total absence of direct xylem flow into maturing seeds at all times, day or night (Fig. 6), while confirming the existence of diurnally reversing flow of water between fruit and plant, and validating the picture of xylem flow predicted for cowpea fruits using the C : N : H_2O modeling exercise summarized in Figure 19.

It remains to be seen whether reversible xylem exchange of water is a regular feature of the physiology of all legume fruits. One might argue, for instance, that it is most likely to be prevalent in and possibly restricted to domesticates with fast-growing large seeds, as exemplified by both *Phaseolus* and *Vigna,* since these are most likely to generate surplus phloem-derived water through massive and rapid intake through phloem. Now that relatively simple assays using xylem mobile dyes such as acid fuchsin are available for testing diurnal and age-related patterns of xylem input by fruits, it should be possible to screen relatively large numbers of species for presence or absence of the trait and then subject species proving positive to further, more rigorous examination.

V. Implications of Fruit Water Balance to the Accumulation of Minerals

Stemming directly from its massive reliance on phloem import, the elemental composition of the dry matter of the legume fruit resembles closely that of the entering phloem stream, being notably high in nutrients present at high level in phloem (e.g., N, P, S, Mg, and K) but low in elements (e.g., Ca, Mn, B, Fe) typically classed as sparingly mobile in phloem but fully mobile in xylem (see Ziegler, 1963; Wiersum, 1966; Bollard, 1970; Epstein, 1972; Pate, 1975; Mix & Marschner, 1976). Indeed, when growing in soils low in any of the latter group of elements, fruits may exhibit specific deficiency symptoms, such as the split seed syndrome shown by certain sweet (alkaloid-free) genotypes of *Lupinus angustifolius* under manganese deficiency (Perry & Gartrell, 1976; Walton, 1978). This last disorder is characterized by critically low (<1 μg ml^{-1}) levels of Mn in the phloem stream to fruits, despite high levels of Mn in foliage supplying assimilates to the reproductive organs (Hocking et al., 1977). Direct xylem import does supplement significantly the fruit's total intake of

Mn (Hocking & Pate, 1977), but, as mentioned previously, developing seeds are unlikely to draw directly from this source.

Fruits of domesticated grain legumes are most likely to be prone to deficiency of elements supplied poorly through phloem, since man has generally selected for fast-maturation and large-seededness, features promoting massive phloem inputs of water and solutes and accordingly reduced xylem import. Any of three physiological strategies might be envisaged to counteract deficiency of predominantly xylem-borne nutrients. First, phloem mobility of the elements in question might be increased in the phloem stream specifically when nutrients are being mobilized from vegetative plant to fruit (see Loneragan, 1982; Hannam et al., 1985) or from pericarp to seed (Hocking & Pate, 1977). There is no really conclusive proof that this happens, but judging from published analyses of phloem exudates, those obtained directly from legume fruits appear to be relatively rich in certain elements, notably calcium (e.g., see Pate et al., 1974, 1984). There are, of course, basic chemical constraints in transporting elements such as iron and calcium while maintaining mobility of phosphorus and other elements, especially at the relatively high pH characteristic of phloem exudates, and this may well limit the extent to which mobilities might be conditionally increased during reproductive development.

Second, and applicable only to fruits developing below ground, supplementation of a fruit's import from the parent plant might take place by absorption of certain mineral elements directly from the soil through the fruit's outer epidermis. The classic case of this relates to calcium in peanut (*Arachis hypogaea*) (Bledsoe et al., 1949), but the hydrophilic nature of the outer epidermis exhibited by other geocarpic grain legume fruits (e.g., see Fig. 14) suggests that it might apply to other species.

Third, and perhaps most importantly, diurnally reversing xylem flow between fruit and parent plant could be readily employed as a means of providing a critically important input of xylem-borne solutes at night. For reasons mentioned earlier, this would require that significant transpiration of the fruit occur at night and that deficits in water balance of the fruit at such times were not fully met by current intake of water by mass flow in phloem. These are the very conditions described earlier (Table 2) for the maturing fruit of cowpea, although detailed budgets for phloem and xylem inputs of mineral elements are not yet available for this species. In species of lupines (*Lupinus albus* and *L. angustifolius*), however, detailed inventories of intake of a range of nutrients have been made on both a daily and longer-term basis. The data are based on the assumption that unidirectional intake takes place by mass flow in both xylem and phloem, and that mineral elements enter at concentrations recorded in analyses of phloem and xylem exudates. The resulting budgets (see Pate & Hocking, 1978; Hocking et al., 1978), based on satisfying the carbon and water requirements of the fruit, indicate that vascular intake during a fruit's life matches to within ±5 to 25% the recorded increments of the elements N, Na, Zn, Fe, Cu, P, K, and S, but accounts for less than one-third of predominantly xylem mobile elements such as Ca and Mn. The anomalies relating to the last two elements would be resolved were it assumed that additional water entered the fruit during times of low phloem intake.

VI. Efficiency of Conversion by Fruits of Imported Organic Solutes into Seed Reserves

Once models have been constructed of the amounts of carbon and nitrogen consumed by the fruit, and the proportional intake of these elements through xylem and phloem, this information can then be combined with organic solute analyses of phloem sap and xylem sap to determine the actual weight of different classes of solutes imported through the fruit stalk (see Pate, 1984, 1985). Of the three species (cowpea, pea, and lupine) thus far investigated, all import sucrose as their major source of carbohydrate, with much lesser amounts of carbon in the form of organic acids and various nitrogenous solutes. As far as the latter are concerned, fruits of all three species import relatively

TABLE 3. Conversion by legume fruits of imported solutes into seed dry matter and protein. Data from Pate (1985).

	Garden pea (*Pisum sativum cv.* Greenfeast)	White lupine (*Lupinus albus cv.* Neutra)	Cowpea (*Vigna unguiculata* cv. Vita 3)
1. *Solutes entering fruits in xylem and phloem (mg/fruit)*			
Sucrose + organic acids	2,404	1,756	3,437
Amides + organic acids	363	384	660
Ureides	30	—	72
Total	2,797	2,140	4,169
2. *Reserve materials laid down in seeds* (mg/fruit)			
Total dry matter	1,930	1,070	3,220
Protein	387	412	719
Oil	19	132	30
Perchloric acid–soluble carbohydrate (starch and sugar)	857	110	1,803
3. *Conversion efficiency by weight of imported solutes to seed components* (%)			
To dry matter	69	50	77
To protein	14	19	17

large amounts of amides and amino acids, especially the amides asparagine and glutamine. In cowpea and pea, however, additional nitrogen is also imported in the form of the ureides allantoin and allantoic acid. The total amounts of each of these classes of compounds imported over the life of these fruits of each species are set out in the first column of Table 3.

At maturity, the seeds of the respective fruits have laid down a certain amount of dry matter, composed in part of measurable amounts of protein, perchlorate-soluble carbohydrate, and oil (item 2, Table 3). Composition varies between the study species, lupine showing the highest percentage of its seed weight as protein and, unlike the other species, having carbohydrate reserves principally as insoluble wall-bound polymers, not as starch.

From these data one can proceed to compare the overall performance of the fruits of each species in terms of percentage conversion by weight of total imported solutes into specific seed reserves or total seed dry matter (item 3, Table 3). The cultivar of cowpea used in the comparison turns out to show the highest conversion index (77%) in terms of conversion of fruit imports to total seed dry matter, compared with 69% in the garden pea cultivar and only 50% in white lupine. As shown in an earlier study (Pate et al., 1977), the mature white lupine fruit carries a large body of irretrievable dry matter in its thick fibrous pod wall, and it is this which principally accounts for low efficiency of the species in conversion of imports to seed dry matter. However, if fruits of the same three species are compared in terms of production of seed protein (item 3, Table 3), white lupine, with a conversion index of 19%, proves to be slightly superior to cowpea (17%) and pea (14%).

Were the above approach to be extended to a wider range of legume species and to be accompanied by detailed studies of fruit anatomy, biochemistry, and physiology, one would begin to understand more clearly how specific structural and functional features interact in shaping the total economy of the fruit. Such information might prove as interesting to study of resource allocation in native legumes in their natural environments as to evaluations of how best to select domesticated species for greater productivity under agricultural conditions.

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References

Atkins, C. A. & A. M. Flinn. 1978. Carbon dioxide fixation in the carbon economy of developing seeds of *Lupinus albus* (L.). Pl. Physiol. (Lancaster) 62: 486–490.

———, J. S. Pate & M. B. Peoples. 1986. Water relations of cowpea fruits during development. *In*: H. Lambers, J. J. Neeteson & I. Studen (editors), Fundamental, Ecological and Agricultural Aspects of Nitrogen Fixation in Higher Plants. Developments in Plant and Soil Sciences, Volume 19.

———, J. Kuo, J. S. Pate, A. M. Flinn & T. W. Steele. 1977. Photosynthetic pod wall of pea (*Pisum sativum* L.). Distribution of carbon dioxide-fixing enzymes in relation to pod structure. Pl. Physiol. (Lancaster) 60: 779–786.

Bledsoe, R. W., D. L. Comar & H. C. Harris. 1949. Absorption of radioactive calcium by the peanut fruit. Science 109: 329–330.

Bollard, E. G. 1970. The physiology and nutrition of developing fruits. Pp. 387–425 *in* A. C. Hulme (editor), The Biochemistry of Fruits and Their Products, Volume 1. Academic Press, London.

Clements, H. F. 1940. Movement of organic solutes in the sausage tree, *Kigelia africana*. Pl. Physiol. (Lancaster) 15: 689–700.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York.

Esau, K. 1965. Plant Anatomy, 2nd edition. John Wiley, New York.

Fahn, A. & M. Zohary. 1955. On the pericarpial structure of the legumen. Its evolution and relation to dehiscence. Phytomorphology 5: 99–111.

Flinn, A. M., C. A. Atkins & J. S. Pate. 1977. Significance of photosynthetic and respiratory exchanges in the carbon economy of the developing pea fruit. Pl. Physiol. (Lancaster) 60: 412–418.

Hannam, R. J., R. D. Graham & J. L. Riggs. 1985. Diagnosis and prognosis of manganese deficiency in *Lupinus angustifolius* L. Austral. J. Agric. Res. 36: 765–767.

Hocking, P. J. & J. S. Pate. 1977. Mobilization of minerals to developing seeds of legumes. Ann. Bot. (London) 41: 1259–1278.

———, ———, C. A. Atkins & P. J. Sharkey. 1978. Diurnal patterns of transport and accumulation of minerals in fruiting plants of *Lupinus angustifolius* L. Ann. Bot. (London) 42: 1277–1290.

———, ———, S. C. Wee & A. J. McComb. 1977. Manganese nutrition of *Lupinus* spp. especially in relation to developing seeds. Ann. Bot. (London) 41: 677–688.

Kuo, J. & J. S. Pate. 1985. Unusual network of internal phloem in the pod mesocarp of cowpea [*Vigna unguiculata* (L.) Walp. (Fabaceae)]. Ann. Bot. (London) 55: 635–647.

Layzell, D. B. & T. A. LaRue. 1982. Modeling C and N transport to developing soybean fruits. Pl. Physiol. (Lancaster) 70: 1290–1298.

Loneragan, J. T. 1982. Mineral nutrition. III. Response of plants to trace elements. Progr. Bot. 44: 92–102.

Mix, G. P. & H. Marschner. 1976. Calcium Umlagerung in Bohnen-Früchten während des Samen Wachstums (Redistribution of calcium in bean fruits during seed development). Z. Pflanzenphysiol. 80: 354–366.

Münch, S. 1930. Die Stoffbewegungen in der Pflanze. Gustav Fischer, Jena.

Muntz, K., A. Rudolph, G. Schleiser & P. Silhenest. 1978. The function of the pericarp in fruits of crop legumes. Kulturpflanze 26: 537–567.

Pate, J. S. 1975. Exchange of solutes between phloem and xylem and circulation in the whole plant. Pp. 451–473 *in* M. H. Zimmermann & J. A. Milburn (editors), Encyclopedia of Plant Physiology New Series, Volume 1—Transport in Plants. I. Phloem Transport. Springer-Verlag, Berlin.

———. 1984. The carbon and nitrogen nutrition of fruit and seed—case studies of selected grain legumes. Pp. 42–82 *in* D. R. Murray (editor), Seed Physiology, Volume 1. Academic Press, Sydney.

———. 1985. Physiology of pea—a comparison with other legumes in terms of economy of carbon and nitrogen in whole-plant and organ functioning. Pp. 279–296 *in* P. D. Hebblethwaite, M. C. Heath & T. C. K. Dawkins (editors), The Pea Crop. Butterworths, London.

——— & A. M. Flinn. 1973. Carbon and nitrogen transfer from vegetative organs to ripening seeds of field pea (*Pisum arvense* L.). J. Exp. Bot. 24: 1090–1099.

——— & P. J. Hocking. 1978. Phloem and xylem transport in the supply of minerals to a developing legume (*Lupinus albus* L.) fruit. Ann. Bot. (London) 42: 911–921.

_____ & J. Kuo. 1981. Anatomical studies of legume pods—a possible tool in taxonomic research. Pp. 903–912 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____, M. B. Peoples & C. A. Atkins. 1984. Spontaneous phloem bleeding from cryopunctured fruits of a ureide-producing legume. Pl. Physiol. (Lancaster) 74: 499–505.

_____, P. J. Sharkey & C. A. Atkins. 1977. Nutrition of a developing legume fruit. Functional economy in terms of carbon, nitrogen, water. Pl. Physiol. (Lancaster) 59: 506–510.

_____, _____, & O. A. M. Lewis. 1974. Phloem bleeding from legume fruits—a technique for study of fruit nutrition. Planta 120: 229–243.

_____, M. B. Peoples, A. J. E. van Bel, J. Kuo & C. A. Atkins. 1985. Diurnal water balance of the cowpea fruit. Pl. Physiol. (Lancaster) 77: 148–156.

Peoples, M. B., J. S. Pate, C. A. Atkins & D. R. Murray. 1985. Economy of water, carbon and nitrogen in the developing cowpea fruit. Pl. Physiol. (Lancaster) 77: 142–147.

Perry, M. W. & J. W. Gartrell. 1976. Lupin 'split seed'. A disorder of seed production in sweet narrow-leafed lupins. J. Agric. Western Australia 17: 20–25.

Summerfield, R. J., J. S. Pate, E. H. Roberts & H. C. Wien. 1985. The physiology of cowpeas. Pp. 65–101 *in* S. R. Singh & K. O. Rachie (editors), Cowpea Research, Production and Utilization. John Wiley & Sons, New York.

Walton, G. H. 1978. The effect of manganese on seed yield and the split seed disorder of sweet and bitter phenotypes of *Lupinus angustifolius* and *L. cosentinii*. Austral. J. Agric. Res. 29: 1177–1189.

Wiersum, L. K. 1966. Calcium content of fruits and storage tissues in relation to the mode of water supply. Acta Bot. Neerl. 15: 406–418.

Woodcock, E. F. 1934. Carpel anatomy of the bean (*Phaseolus vulgaris* L.). Annual Rep. Michigan Acad. 20: 267–271.

Ziegler, H. 1963. Verwendung von ^{45}Calcium zur Analyse der Stick Stoffversorgung wachsender Früchte. Planta 60: 41–45.

LEGUME SEEDS—THE STRUCTURE:FUNCTION EQUATION

J. Van Staden, J. C. Manning and K. M. Kelly[1]

ABSTRACT

The structure of a biological organ represents the phenotypic expression of selection acting within the constraints imposed by the developmental processes involved. The seed is a multifunctional structure derived from multiple tissues of diverse origin and ploidy. Various strategies have evolved that facilitate the seed functioning in the most efficient manner, which ensures genetic survival of the species. The legume testa is, with few exceptions, characteristic of the family: accessory structures have evolved to improve the environmental exploitation by various species. Color polymorphism and various physical, phenological, and chemical strategies are important in avoiding or reducing predation, particularly by bruchids, but also by potential avian or ungulate dispersers. The development of an impermeable testa and associated seed dormancy is a common strategy. Testa structure and the functioning of the pleurogram and hilar fissure are instrumental in permitting desiccation and simultaneously preventing imbibition. Dry seeds maintain contact with ambient environmental events via the lens, which is sensitive to specific germination cues, including fluctuating temperatures and percussion. The structure of the lens is related to its role in imbibition and the enhancement of seedling vigor. All aspects of seed structure are linked inextricably to seed functioning. An understanding of seed structure cannot be achieved by adopting a unidirectional approach but only by an awareness of the multidimensional significance during its development and evolution.

I. INTRODUCTION

The structuring of a biological organ represents the phenotypic expression of selection acting within the constraints imposed by the developmental processes involved. A strictly channeled ontogenetic sequence leaves little opportunity for extensive modifications within the sequence. However, one open to environmental input will be modified more readily during its operation and during evolution of the organism without deleterious results on the epistatic interactions between the genes. Thus, some developmental sequences are more sensitive to external pressures than others, and the morphological result of pressures acting also on the physiology, biochemistry, and cytology of an organ may or may not be somewhat restricted in certain directions. Therefore, the structure of a seed is determined by the superimposing of contemporary selective influences onto a framework determined historically and which may be adapted more or less readily.

The selective influences acting on a seed are particularly varied (Fig. 1). The seed is a multifunctional structure derived from multiple tissues of diverse origin and ploidy, each of which is influenced by the other, and each of which has a somewhat different genetic framework from which to diversify. Consequently, conditions experienced by the maternal plant may be manifest in the embryo sac, embryo, and associated seed structures. Furthermore, adjacent ovules influence each other's environment, even to the demise of some. The developing seed requires adequate nutrients and water, protection from desiccation, and space in which to grow. Once mature, it is able to participate independently in the life of the plant and is liable to selective pressures of a different nature. The seed must be successful in dispersal and settlement in an appropriate environment, the survival of adverse conditions, and the rapid optimization of favorable conditions on their appearance. This must be achieved despite the potentially fatal presence of predators and competitors at

[1]UN/FRD Research Unit for Plant Growth and Development, Department of Botany, University of Natal, Pietermaritzburg 3200, Republic of South Africa.

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MONOGR. SYST. BOT. MISSOURI BOT. GARD. 29:417–450. 1989.

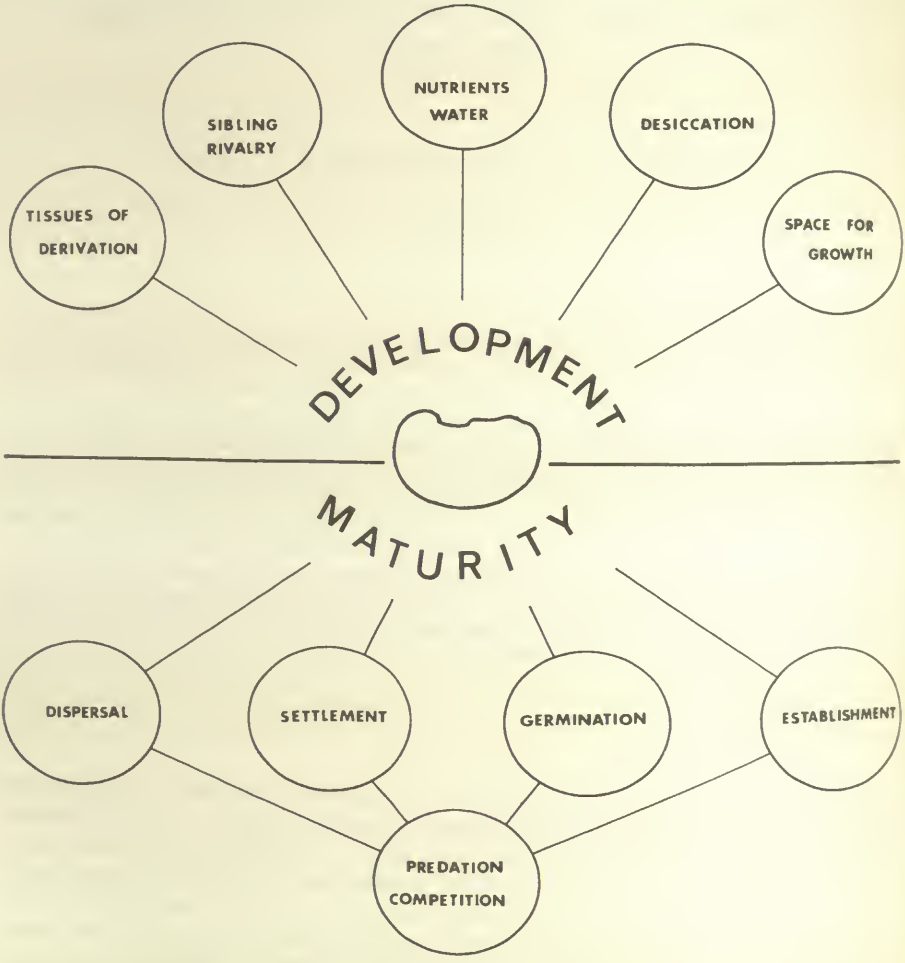


FIGURE 1. Sources of selection pressures on seeds.

all stages. To this end, various strategies have evolved that facilitate the most efficient functioning of the seed to ensure genetic survival of the species. The appropriate solution, however, is not necessarily that which is *potentially* most successful but the compromise which is *effectively* most successful. In addition, the solution is dynamic. Seed function is inextricably linked to seed structure, and seed functioning is a multiphase sequence that begins long before the appearance of the mature seed. Thus the actualized seed structure is a function of multiple influences at all stages of its development. Consequently, a study of the seed structure:function relationship must involve a study of the life history of the seed.

It is important to emphasize just how integrated a unit the legume seed is. An understanding of its structure cannot be achieved by adopting a unidirectional approach but only by being aware of the multidimensional significance during its development and evolution (Fig. 2). A brief survey of seed structure in the family is followed by an appraisal of the various influences on the seed and a consideration of some of the solutions, bearing in mind the constraints to unlimited modification. What is evident is the relative consistency of the basic structure of the papilionoid and caesalpinioid–

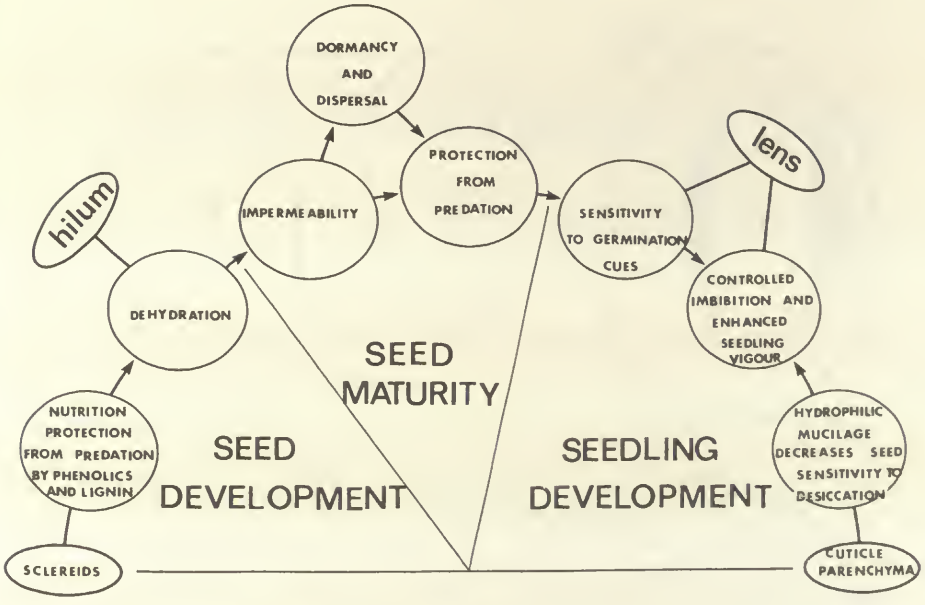


FIGURE 2. The progression of functions of the legume testa.

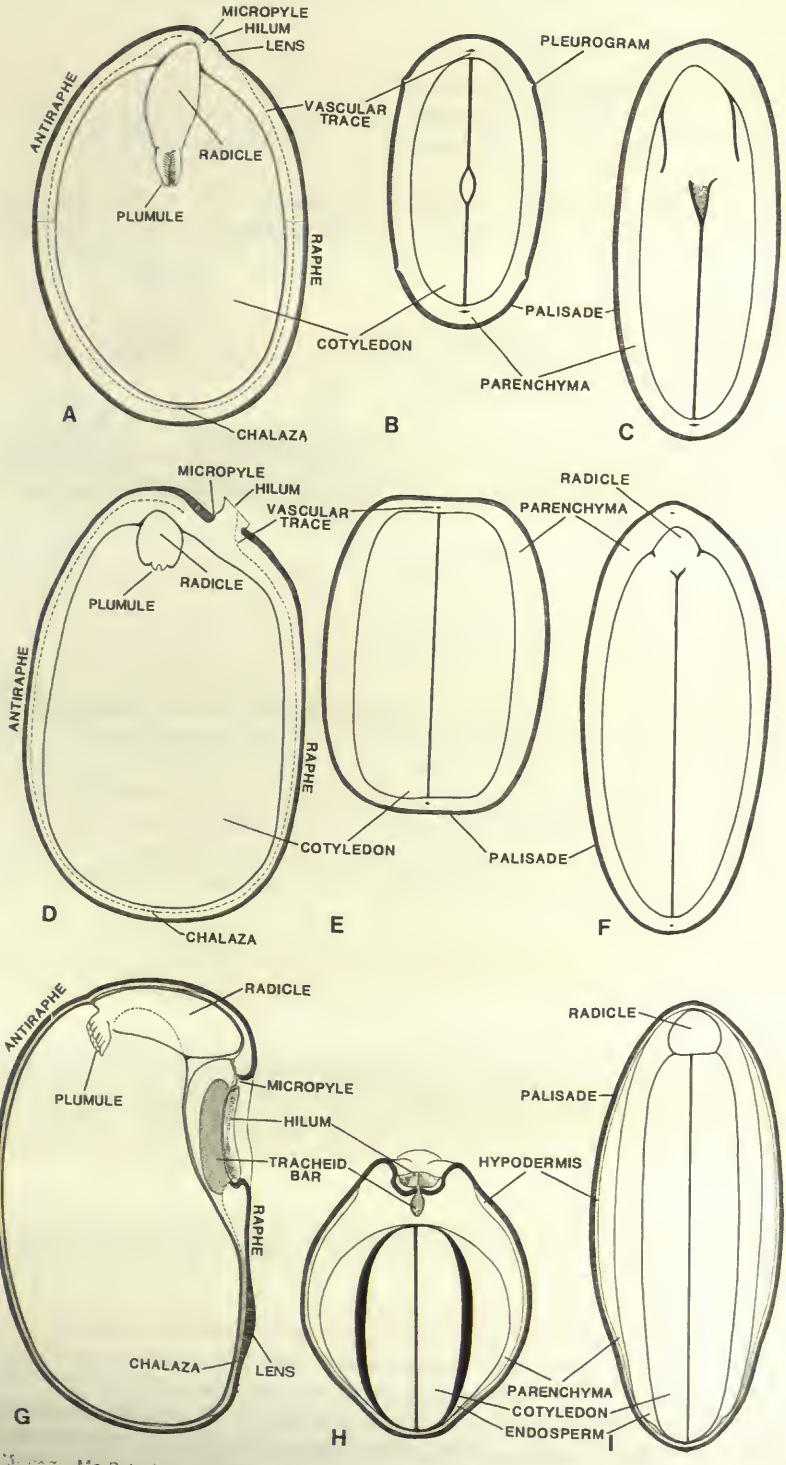
mimosoid seeds respectively. This relatively conservative structure represents a very successful framework from which many particular adaptations emerge, and the success of the family can be attributed in part to the relative versatility of the legume seed in meeting environmental and phylogenetic challenges. Unfortunately, the physiological and associated ultrastructural peculiarities of the embryo itself have been studied little, and their neglect constitutes a major omission in the understanding of seed functioning. Nevertheless, what is known of the legume seed structure and function is sure to stimulate awareness of the importance of an integrated approach to plant functioning.

II. Seed Structure

Seed structure in the Leguminosae has been the subject of numerous studies, including those of Corner (1951) and Gunn (1981a, b), which constitute a useful introduction. In this section the structure of the legume seed will be reviewed, with particular reference to structures whose role in seed functioning is considered later in the review. Some seed characters have diagnostic value at the subfamily level and are discussed below.

THE CAESALPINIOID–MIMOSOID SEED

These seeds are usually derived from anatropous ovules which maintain this condition in most representatives (Kopooshian & Isely, 1966). Ovules are bitegmic, and the outer integument alone participates in the formation of the testa. The embryonic axis is straight in nearly all Mimosoideae (rarely slightly deflexed in *Calliandra* and some *Adenanthera;* Fig. 3A, C) and straight to curved *(Bauhinia)* in the Caesalpinioideae (Fig. 3D, F) (Gunn, 1981a). The plumule usually is poorly



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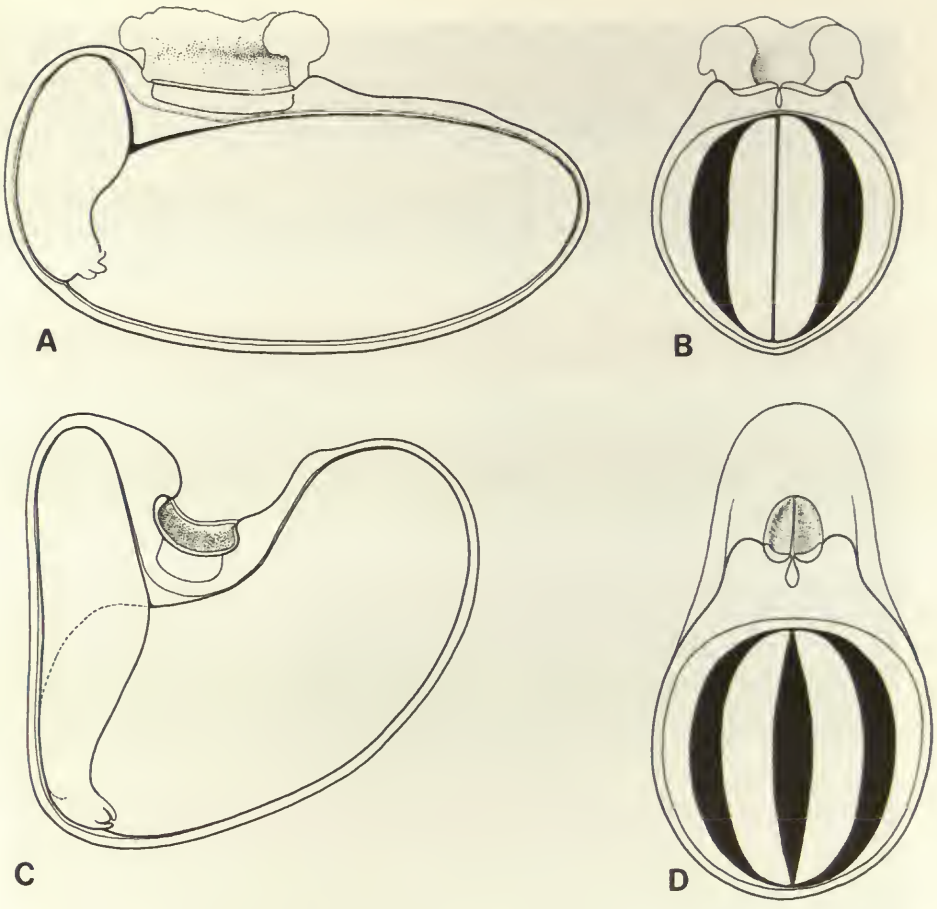

FIGURE 4. Seeds of Papilionoideae.—A, B. *Liparia sphaerica*.—C, D. *Crotalaria capensis*.

developed in the Caesalpinioideae (Fig. 3D, F) and rudimentary to well developed in the Mimosoideae (Fig. 3A, C).

Seeds (excluding the hilum) are bilaterally symmetrical, with the chalaza and hilum at opposite ends of the seed and with raphe and antiraphe lengths essentially equal (Fig. 3A, D). The hilum is contiguous with the tip of the radicle, sub-basal, small, punctiform to shortly oblong (Figs. 4B, 5B) (crescentic in *Bauhinia*), and simple, with the surface composed of one layer of palisade cells. The micropyle is microscopic and slit-like in most Mimosoideae (Fig. 6B); punctate, slit-like, or Y-shaped in the Caesalpinioideae (Manning & Van Staden, 1987a). The lens is insignificant to very obvious and is usually discolored, and a slightly raised oval, circular, or flattened structure is present along the raphe near the hilum (Figs 5B, 6B). The vasculature consists of an unbranched vascular bundle that forms a loop extending almost completely around the seed (Fig. 3) (some exceptions in *Bauhinia*; [Corner, 1951; Kopooshian & Isely, 1966]), passing outwards in the region

←FIGURE 3. Seeds of Leguminosae—A–C. *Acacia farnesiana*.—D–F. *Schotia brachypetala*.—G–I. *Sophora japonica*. (A,D,G median longitudinal section through raphe-antiraphe. B,E,H transverse section. C,F,I median longitudinal section normal to raphe-antiraphe.)

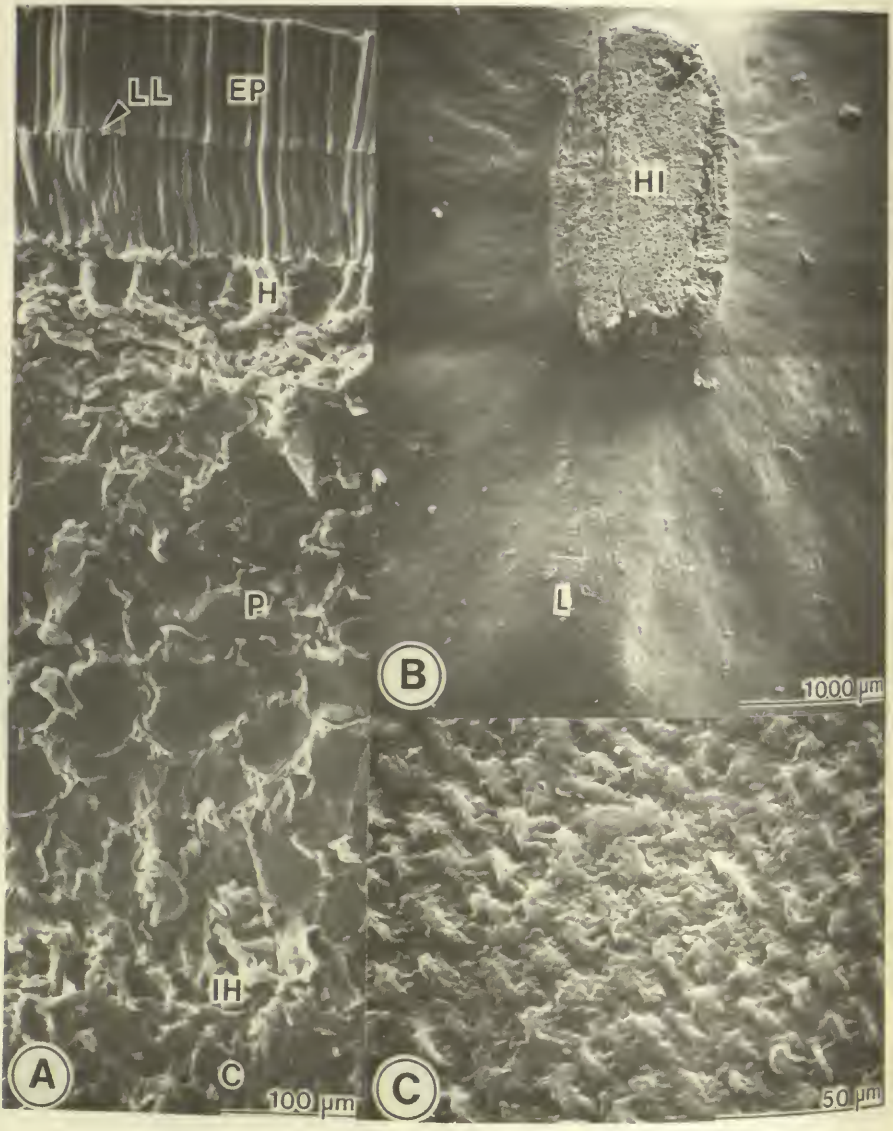

FIGURE 5. Seed of Caesalpinioideae. *Schotia brachypetala*.—A. Testa.—B. Hilar region.—C. Testa surface. EP epidermal palisade, LL light line, H hypodermal hourglass cells, P parenchyma, IH inner epidermal hourglass cells, C cotyledons, HI hilum, L lens.

of the lens to lie just beneath the epidermal palisade and then arching inwards again beyond the lens to its usual position in the parenchyma. It is essentially nonfunctional between hilum and lens but much more prominent beneath and beyond the lens (Hanna, 1984). Unlike the phloem, the xylem is not crushed (Hanna, 1984).

The testa is smooth, seldom pitted or wrinkled (Figs. 5C, 6A) (Trivedi et al., 1979; Bragg & Bridges, 1984; Manning & Van Staden, 1987a). A pleurogram is often present and complete in the

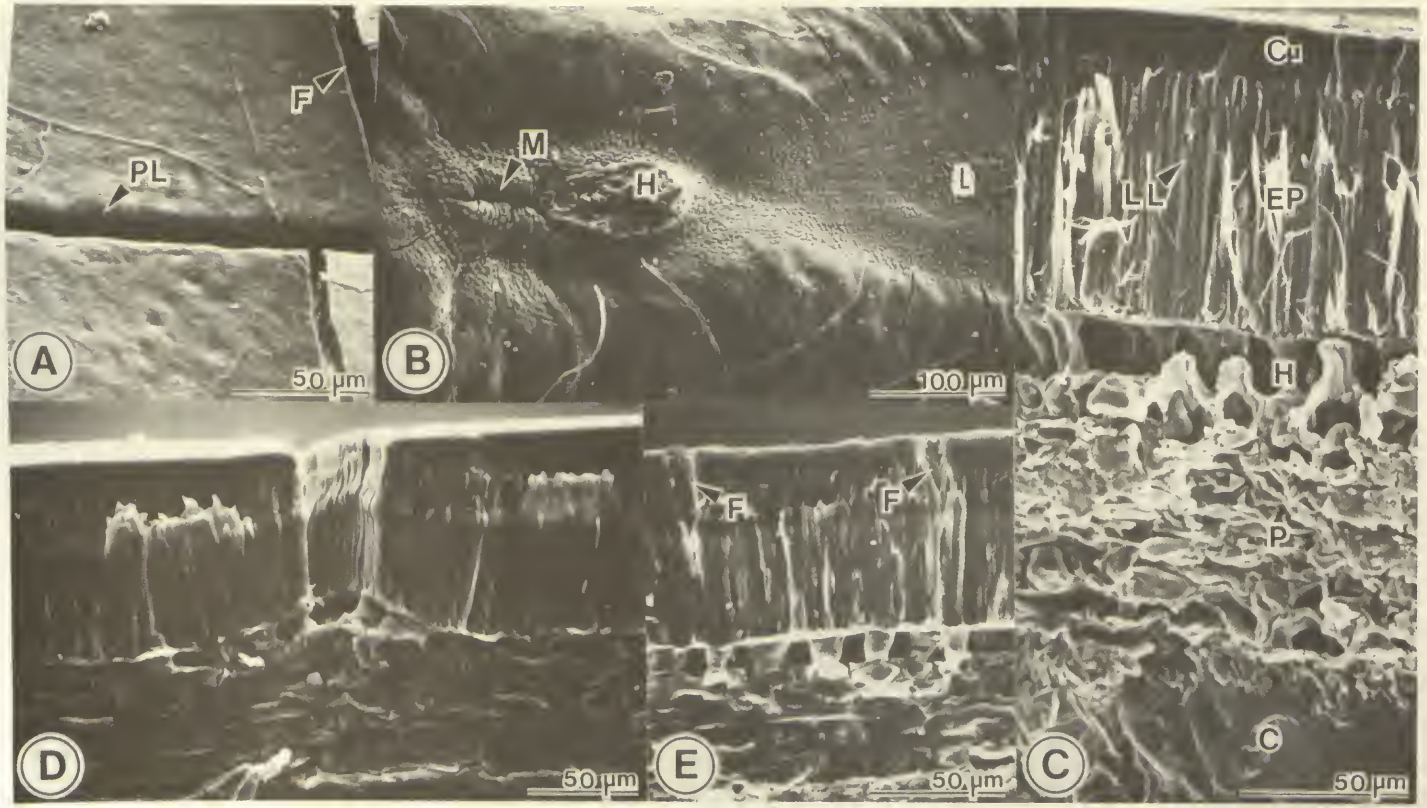

FIGURE 6. Seed of Mimosoideae. A–C. *Albizia adianthifolia*. D, E. *Entade spicata*.—A. Pleurogram (PL) and fracture lines (F) are shown.—B. Testa showing the position of the micropyle (M), hilum (H), and lens (L).—C. Testa with cuticle (Cu), epidermal palisade (EP), hourglass cells (H), parenchyma (P), and cotyledons (C).—D. Testa, pleurogram fissure penetrates completely through the epidermis.—E. Testa, the fracture lines (F) penetrate only to the light line.

Mimosoideae (Gunn, 1984) (Fig. 6A), rarely present and often open in the Caesalpinioideae (Corner, 1951; Gunn, 1981a). It is visible in immature seeds as a localized area where the epidermal cells are shorter than the cells from other areas, and the area at the base of the shorter cells is filled in by parenchyma cells derived from periclinal divisions in the young hypodermal cells (Robbertse, 1973). In the mature seeds, the pleurogram is visible as a fissure extending completely through the epidermis (Fig. 6D). Fracture lines are present or absent, and they penetrate through the cuticle or through the outer ends of the palisade, rarely completely (Fig. 6A, B). The cuticle ranges from thin (1–5 μm) (Fig. 5A) to very thick (14–40 μm), and the epidermal cells penetrate as cones a little way into the cuticle (Fig. 6C). Species with a thinner cuticle (2.5 μm or less) have rugose or similar cuticular sculpturing (Fig. 5C), while those with a thicker cuticle (10 μm or more) have a smooth surface (Fig. 6A); therefore, cuticular sculpturing presumably is related to the proximity of the underlying epidermis.

The epidermis is formed from the outer epidermis of the outer integument and consists of radially elongated (as long as 235 μm in *Schotia afra*) palisade sclereids (Figs. 5A, 6C). Generally palisade thickness is greatest in the Caesalpinioideae. Radial elongation and thickening of the epidermis begins early in seed development, and thickening takes the form of longitudinal ridges in the upper part of the cell (Suri & Deshpande, 1981). A light line is present near the center of the cells (Figs. 5A, 6C), rarely in the apical third. The hypodermal cells are differentiated into hourglass-shaped cells (except at the lens) (Figs. 5A, 6C) or, rarely, are undifferentiated (some species of *Acacia* and *Caesalpinia*), and sometimes tuberculate *(Schotia)* (Fig. 5A). Below the hypodermis are few to many layers of parenchyma, bounded internally by the inner epidermis of the outer integument, which may be differentiated into an inner layer of hourglass-shaped cells *(Entada, Delonix, Schotia)* that are tuberculate in *Schotia* (Fig. 5A). Beneath the outer integument are crushed remnant layers of the inner integument and nucellus; and below that is endosperm, usually abundant (Isely, 1955). Epidermal cells of the lens may be longer than those elsewhere, and the hypodermis in this region is undifferentiated and replaced by thin-walled parenchyma (Hanna, 1984). The start of the lens in *Acacia* is indicated by a multiseriate channel of thin-walled hypodermal parenchyma located directly below the palisade. The channel bifucates beneath the lens, and the arching vascular bundle passes between the two channels thus formed and then directly below the remaining monolayer of hypodermal parenchyma. At the end of the lens the two channels and joining monolayer disappear, and the vascular bundle passes sharply inward to its normal position in the parenchyma (Hanna, 1984).

THE PAPILIONOID SEED

These seeds are derived from campylotropus ovules, although the nature and degree of bending varies (Kopooshian & Isely, 1966). Ovules are bitegmic, and the outer integument alone participates in the formation of the testa. The embryonic axis is influxed (one or more bends; Fig. 4A, C) and very rarely straight (genera in the Bossiaeeae, Brogniartieae, Dalbergieae, Dipterygeae, Euchresteae, Phaseoleae, Sophoreae, Swartzieae [Kopooshian & Isely, 1966; Gunn, 1981a]; Fig. 3G). Seeds (excluding the hilum) are bilaterally asymmetrical (rarely bilaterally symmetrical), with the chalaza and hilum closely approximate and with the raphe shorter than the antiraphe (Fig. 3G). The hilum is lateral, seldom apical, short to long (3/4 of seed circumference), variously shaped, usually complex and bearing a median hilar fissure, and characterized by a clean abscission surface (unless obscured by an epihilum).

Seed abscission usually occurs at the funicle–ovule junction such that a single layer of elongate funicular cells remains adnate to the hilar palisade and forms the counter-palisade (Fig. 7A) (absent in *Erythrina* spp.; Manning & Van Staden, 1985) (Fig. 8C). Other funicular remains may be present as an epihilum obscuring the hilum or as a rim-axil encircling the hilum (Lackey, 1981) (Fig. 4A, B).

Immediately beneath the hilar fissure is a bar of tracheoids (Fig. 7A, E) (sometimes absent in *Swartzia*), usually oriented normal to the epidermal cells and rarely parallel to them (Lersten, 1982). In cross section the tracheid bar is pyriform, pointed to the hilar fissure, and extends from near the micropyle to the site of entry of the parental vascular connection (Fig. 3A, G). Differentiation of the tracheid bar occurs late in seed development and coincides with final secondary wall deposition in the hilar palisade (Manning & Van Staden, 1985; Fig. 7C). At the raphe end of the hilum the tracheid bar invariably contacts the vascular bundle of the seed or its sheath (Lersten, 1982).

The micropyle is microscopic and generally of two forms: a Y-shaped fissure, with the fork directed towards the hilum and almost invariably separate from it; and a punctate aperture almost invariably included within hilar tissue (Manning & Van Staden, 1987a). The lens is usually discernible and usually close to the hilum along the raphe (Fig. 3G) or as far removed as opposite the hilum.

There is much variation in the vasculature, in contrast with the caesalpinioid–mimosoid seeds (Corner, 1951, 1976). The vascular bundle may extend as an almost complete loop along the raphe and beyond the chalaza along the antiraphe. The postchalazal bundle may be simple or branched. In other species the bundle extends along the raphe to the chalaza (Figs. 3G, 8E). In addition, two recurrent vascular bundles may be present, issuing from the main bundle near the tracheid bar and extending toward the micropyle beneath the hilum. These also may be branched. In neotenous or overgrown seeds, the vasculature is well developed and very complex, and stomata are characteristically present on the testa (Stirton, pers. comm.). These features are essential in such moist, transpiring seeds, which do not undergo a phase of dehydration.

The testa usually is unsculptured to the naked eye, rarely tuberculate or ridged. A pleurogram and fracture lines are absent. The cuticle is thin (less than 5 μm, rarely thicker) (Manning & Van Staden, 1987a) and variously sculptured (Fig. 7D), rarely laevigate (Lersten, 1981). The epidermis is formed from the outer epidermis of the outer integument (Fig. 8A, B) and consists of radially elongated palisade sclereids (usually 50–100 μm long) (Figs. 7B, 8D). Radial elongation and thickening of the epidermis begins early in seed development (Fig. 7A–C), and thickening takes the form of longitudinal ridges in the upper part of the cell (Reeve, 1946; Sterling, 1954; Manning & Van Staden, 1985). A light line is usually present in the outer quarter of the cells, immediately below the cuticle (Fig. 7B). The hypodermal cells are differentiated into hourglass-shaped cells (Figs. 7B, 8D, E) except at the lens; (Figs. 7B, 8D, E), which are sometimes tuberculate *(Millettia, Abrus)*. Beneath the hypodermis are a few layers of parenchyma, much compressed in most species (Fig. 7B), and the inner epidermis of the outer integument is never differentiated into hourglass-shaped cells. In the circumhilar region, the outer one or more rows of parenchyma undergo some radial elongation and differentiate into hourglass-shaped cells (Fig. 8D). Beneath the outer integument are crushed remnant layers of the inner integument and nucellus, which are very inconspicuous; and below that is a reduced endosperm (insignificant in Vicieae and Phaseoleae: Kopooshian & Isely, 1966; Gunn, 1981a). The lens matures rapidly and the light line is present in this region before developing elsewhere. Epidermal cells at the lens are longer than elsewhere and somewhat bowed under tension; the hypodermis may be parenchymatous, as in *Trifolium* (Hagon & Ballard, 1969), or well-differentiated into elongated palisade osteosclereids as long as or slightly longer than the associated epidermal palisade, as in *Indigofera* (Fig. 8E).

III. Seed Function

Seed development

The Testa

Seed development involves a complex of physiological processes, and the testa:embryonic relationship is one of great importance. The testa:embryo interaction imposes physiological constraints

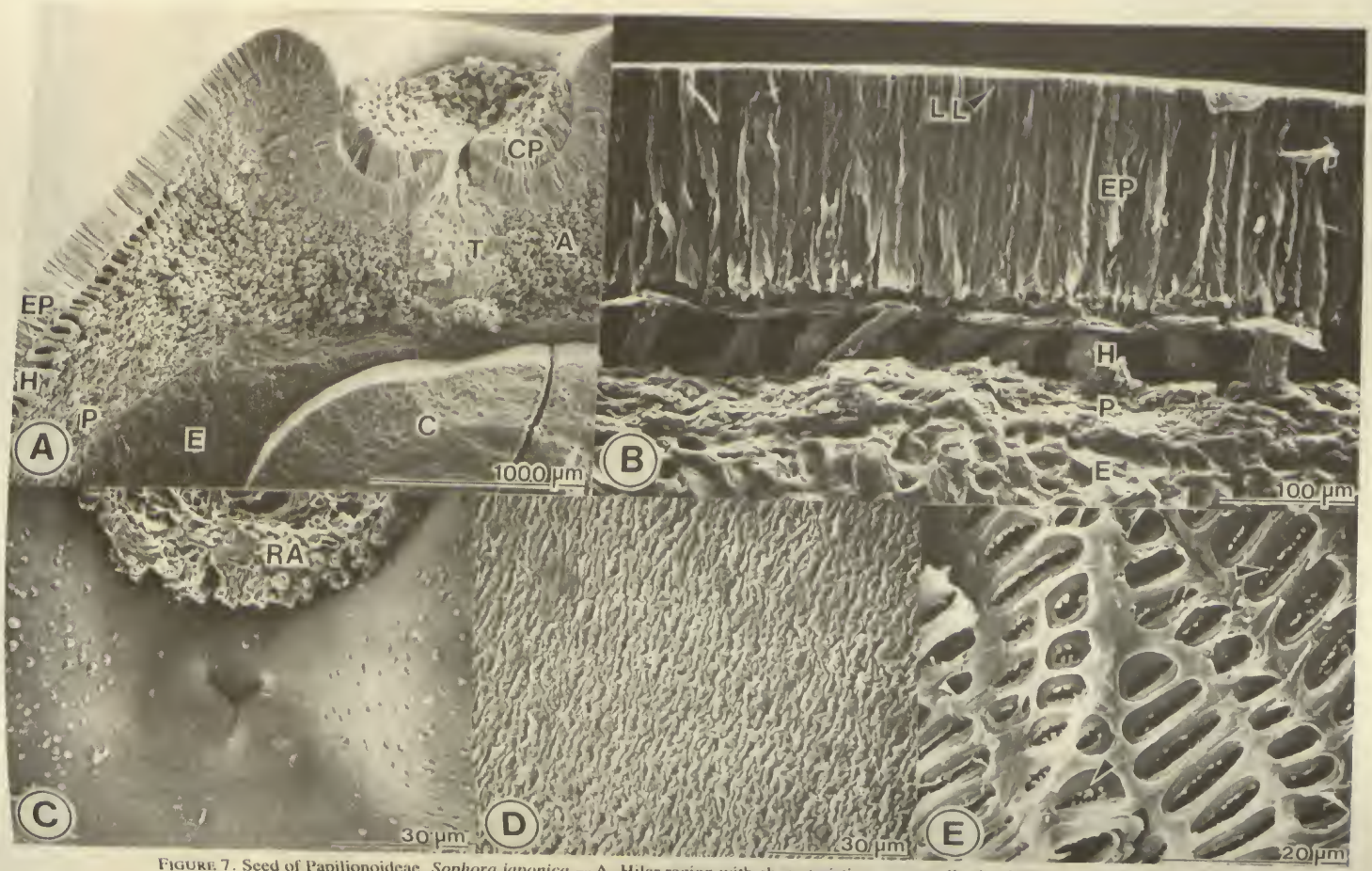

FIGURE 7. Seed of Papilionoideae. *Sophora japonica*.—A. Hilar region with characteristic counter palisade (CP), epidermal palisade (EP), hypodermal hourglass cells (H), tracheid bar (T), parenchyma (P), and endosperm (E). Cotyledons (C) also present.—B. The testa in cross section showing the epidermal palisade (EP), hourglass cells (H), parenchyma (P), endosperm (E), and light line (LL).—C. Micropyle, rim aril (RA).—D. Testa surface.—E. Tracheids from tracheid bar showing pit vestures (arrowed).

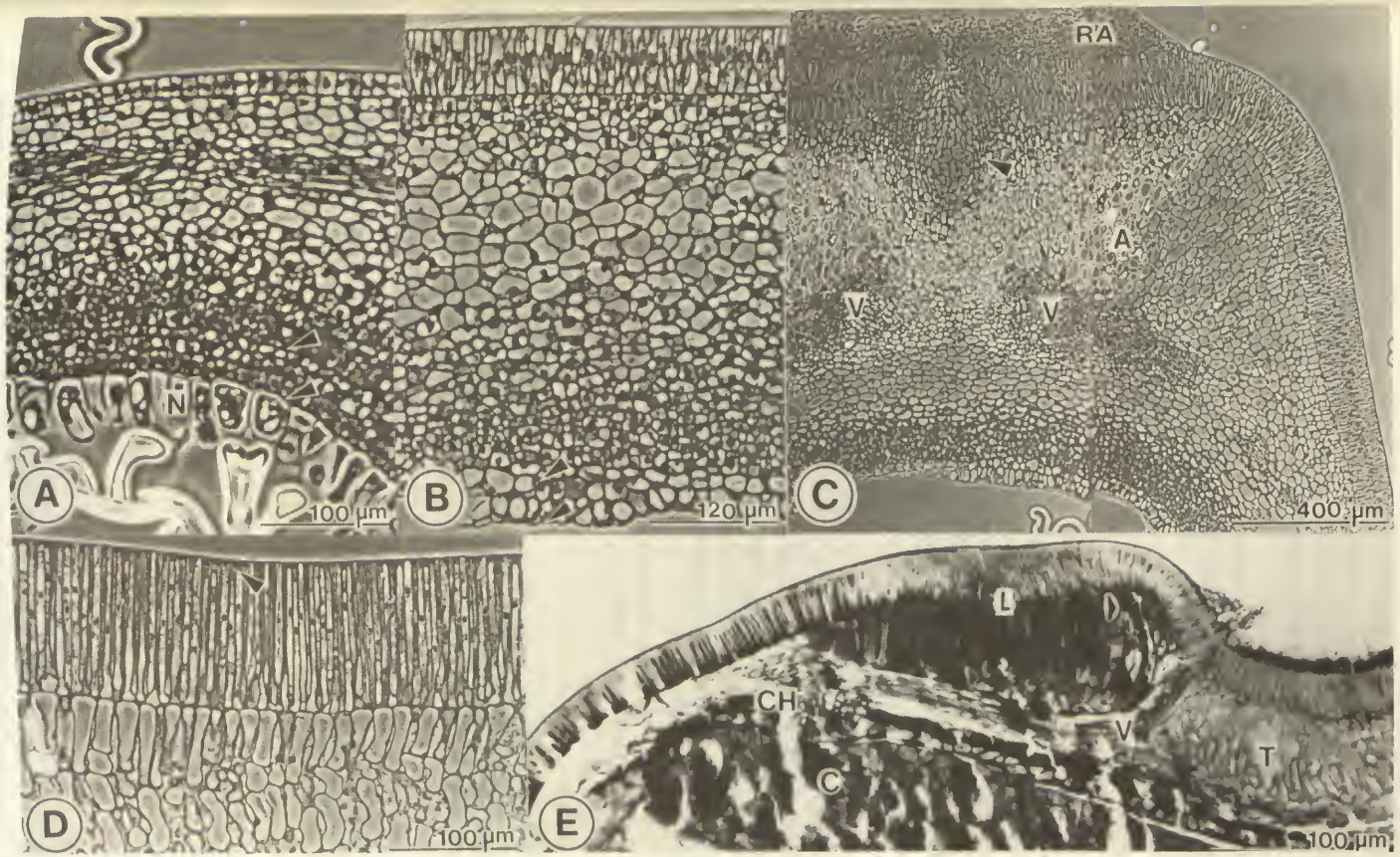

FIGURE 8. Seed development in Papilionoideae. A–D *Erythrina lysistemon*. E. *Indigofera erecta*.—A. Testa opposite hilum. The epidermal cells are slightly elongated and the hypodermal cells are undergoing periclinal division. The inner integument (arrowed) and part of the endosperm (N) are visible. (Seed half-size).—B. Testa near hilum. The epidermal cells are undergoing radial elongation. The inner integument (arrowed) is disrupted. (Seed half-size).—C. Hilar region. Abscission from the funicle is complete and the rim aril remains. The epidermis and hilar palisade is continuous but a counter palisade is lacking. The differentiating tracheid bar (arrowed), recurrent vascular bundles (V), and aerenchyma (A) are well-defined. (Seed 3/4-size).—D. Mature testa near hilum. The palisade cells have a thickened outer tangential wall and the light line (arrowed) is visible immediately below it. The hypodermal cells are differentiated into osteosclereids.—E. Mature lens. The epidermal palisade is thicker at the lens and the cells are somewhat bowed. The underlying hypodermal cells are differentiated into elongate palisade osteosclereids. The vascular bundle extends from the hilum and tracheid bar to the chalaza, passing immediately beneath the lens.

on seed size and determines the extent to which the genetic potential of the seed is realized (Hedley & Ambrose, 1980). The literature suggests that the mechanism whereby testa:embryo effects are mediated involves the growth regulator abscisic acid (Van Staden et al., 1987). The testa of *Pisum sativum* acquires reserves of starch and protein which are mobilized as the testa and embryo mature (Murray, 1979). Mineral reserves also are mobilized from testa to embryo with varying degrees of retention (Hocking & Pate, 1977). Furthermore, during accumulation of assimilates by developing seeds, solutes are transferred from tissues of maternal origin to embryonic tissues and must pass to the testa and then to the embryo (Tanner, 1980; Wolswinkel & Ammerlaan, 1983, 1984). Experiments with *Glycine max* (Thorne & Rainbird, 1983), *Phaseolus vulgaris* (Patrick, 1983), *Pisum sativum* (Wolswinkel et al., 1983), and *Vicia faba* (Wolswinkel & Ammerlaan, 1983) demonstrated that unloading sucrose (quantitatively the most important solute transported into developing legume seeds) and amino acids from the testa is an active process.

The phloem bears most translocated substances from the plant to the seeds (Hocking & Pate, 1977; and see Pate, this volume). However, transfer cells do not accompany the phloem from the funiculus into the testa (Hardham, 1976). Since transfer cells are of paramount importance in loading translocated substances into the phloem, their absence from the tissues of the testa should facilitate unloading of translocated metabolites into the cells of the testa (Murray, 1979). Movement of assimilates from the testa to the embryo is generally apoplastic (Wolswinkel & Ammerlaan, 1985). It is becoming clear that certain metabolites received by the testa from the phloem (including sucrose as well as phosphorous and nitrogen compounds) are radically altered before their transmission to the embryo sac liquid and embryo. Phloem unloading in the testa is probably an important site for the hormonal control of seed growth (Thorne, 1981). In addition, the testa seems to play an important role in hormonal inactivation during seed maturation. During seed desiccation and germination in *Phaseolus vulgaris*, the gibberellin catabolite, which accumulated in the testa during seed development, as well as the products arising from it, are transported from the testa to the embryo (Sponsel, 1983). At present the physiological significance of this is unclear.

Clearly, metabolic and structural changes of considerable importance take place in the cells of the testa during seed development, and the testa is implicated in the control of embryo processes during development and germination by regulation of incoming solutes and by hormonal influence.

The Endosperm

The endosperm in some legumes is a transient tissue present only during the early phases of seed development. It is generally considered as a source and regulator of food reserves to the developing embryo, acting as an intermediate between the maternal and embryonic plants. In order to fulfill this function, the endosperm must act as a strong nutrient sink during the early stages of its existence, a function that it gradually loses. Hormones play an important role in this regard, particularly the cytokinins, which act as nutrient mobilizers (Van Staden et al., 1987). The cytokinins and gibberellins necessary for embryo growth are transported from the parent plant and accumulate in the transient endosperm and then are transported to the embryo in relation to its requirements.

DISPERSAL

The Importance of Dispersal

Next to pollen transport, seed dispersal is the single most important factor promoting gene flow in plants. In addition, the flux of diaspores into and out of a unit of habitat determines the potential population of that habitat. Dispersal is a means whereby an established and stabilized population can maintain itself, and an expanding population can extend its range and increase its size (Harper,

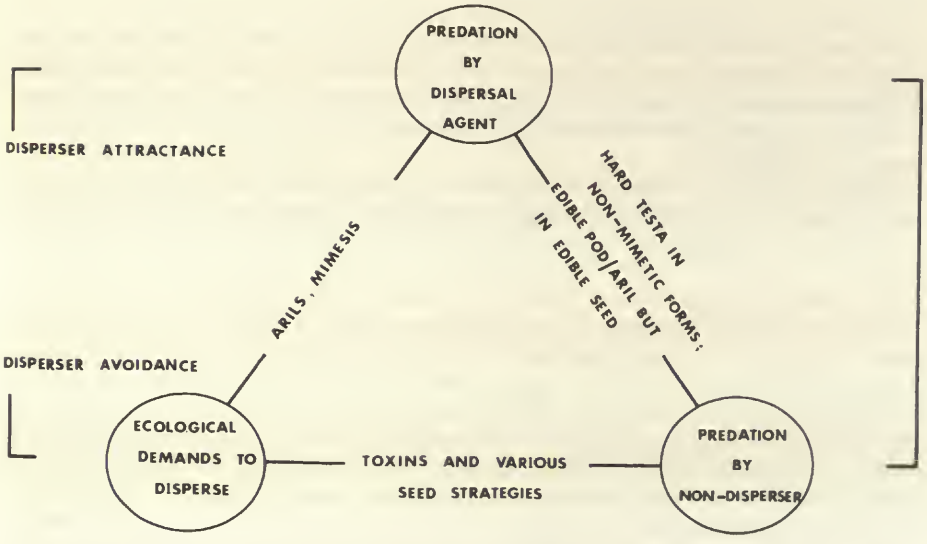

FIGURE 9. The dynamic interaction between attractance and avoidance of a biotic dispersal agent by a seed.

1982; and see Sackville Hamilton, this volume). These two situations are part of the same system, and the significance of dispersal in the life cycle of a plant must therefore depend on the spatial and temporal heterogeneity of its environment (Harper, 1982). Finally, dispersal is a means of escape from predation (Fig. 9). Just as the dispersal habit of a species reflects the selective advantages that dispersive qualities have conferred in the past, the present dispersal characteristics of the species contribute to determining the present ecological range and population size.

If a species is dependent for survival solely on the replacement *in situ* of every individual that dies, it is necessary only for seeds to fall to the ground. However, every species not having a niche in climax vegetation is doomed to local extinction and is in this sense a fugitive species. Some species are more fugitive than others. The more frequently a species experiences the cycle of invasion-colonization-suppression-extinction, the greater the importance of dispersal, as a mechanism of escape, and of a high intrinsic rate of natural increase (Harper, 1982). Fortunately, large seed number, small seed size, and high dispersability readily go hand in hand. Species occupying early places in succession may have less need for substantial food reserves in their seeds than forms establishing in closed, shaded communities. In pairs of related species occupying open and closed communities, it is normal to find the larger-seeded species in the more closed community (Salisbury, 1942). Another well-known ecological correlation is that between large seeds and maritime habitat, and the seeds of *Entada pursathea* (40–60 mm diam.) are no exception. Seed size is thus in part a function of ecological situation, as well as a mode of dispersal.

Dispersal Strategies

Dispersal may be both directed and undirected, and the diaspores both adapted or nonadapted. Browsing animals can swallow diaspores with the foliage and evacuate them intact. In ruminants, this dispersal can hardly be separated from directed, intentional gathering of adapted diaspores. Leguminous herbs (e.g., *Trifolium* or *Indigofera*) with small pods and hard seeds can withstand digestion to some extent.

Dispersal starts with the presentation of the dispersal units (if possible at the right time and place)

as a preparation, including the provision of physiological and structural protective devices. After travel comes settlement (not always by mere deposition) and germination. Only then does the problem of establishment arise. All aspects intermingle and may change in importance during succession and evolution so that traits relating to other spheres of life occur in the dispersal organs. In addition, the existing genotype and phenotype place constraints on the evolution of structural and physiological specialization.

Adapted diaspores are common in the Leguminosae. A distinction between seeds and fruits as the units of dispersal is in many cases ecologically superfluous. However, since fruits in the Leguminosae are treated separately (see Pate, this volume), our discussion is restricted to seeds (Fig. 10). Adaptation for dispersal in seeds of legumes most commonly concerns elaboration of funicular or other extratestal structures. However, the testa may be modified in some species. Initially the seed dominates functionally in dispersal, and the fruit in this phase is a follicle, a container for seeds which possess auxilliary organs. Later fruits evolved in many directions as units of dispersal (see Augspurger, this volume). The influence of nondehiscence of the fruit may be marked, and induced regressive reduction of the testa (as in many Dalbergieae) should not be confused with plesiomorphy. The neotenous seeds of some tropical Sophoreae represent a similar morphological result from different ecological constraints: the very hard, woody pods enclose the rapidly growing seeds in a spongy covering and dehisce to release the large seeds with undifferentiated testa which continue growing and germinate rapidly (e.g., *Alexa, Dussia,* and *Castanospermum;* Stirton, pers. comm.).

Arils in the Mimosoideae occur in some species of *Acacia* and all species of *Pithecellobium* and are functional in ornithochory and other forms of zoochory. A combination of red and black on a diaspore is attractive to birds (Turcek, 1963), and many ornithochorous forms have brilliantly and often contrastingly colored diaspores, often with a predominance of red. In *Acacia cyclops* and *A. falcata* the black seeds are surrounded by a bright red, juicy funicle; in *Pithecellobium* the black seeds are partly enclosed in a reddish aril.

Many ornithochorous species of *Acacia* bear swollen funicles, which may be very long, enabling the seeds to hang from the dehisced pods. Arils occur in 14 caesalpinioid genera *(Afzelia, Copaifera, Sindora).* Some species of *Schotia,* however, have an ill-differentiated parenchymatous testa not induced secondarily by occlusion.

The Papilionoideae show a different seed development, aimed more in the direction of dormancy and help from the pericarp. This is presumably associated with the invasion of less tropical environments and the assumption of the herb and shrub habit. However, arils occur in a number of genera in the Sophoreae. In the primitive tropical papilionoid *Swartzia prouacensis,* the seed itself is still in the arillate phase of exposed seed and has developed a bond with bats, whereas other species employ the same device for ornithochory. Its chiropterochorous adaptations consist of a fairly large seed, a drab white aril (unlike the gaudy seeds of many ornithochorous species), and an immensely long funicle up to 3 m in length. Although arils are commonly functional in ornithochory, they are also implicated in other forms of zoochory. Chiropterochory in *Swartzia* has been mentioned, and the waxy arils of *Sindora* are especially attractive to rodents (van der Pijl, 1982).

Myrmecochorous plants (see McKey, this volume) have been described from many parts of the world (Sernander, 1906; Nordhagen, 1959; Bresinsky, 1963; Berg, 1975; van der Pijl, 1982). Doyle (this volume) discussed the structure, nature, and distribution of the ant-attracting structures (elaiosomes) on legume seeds.

A further aspect of endozoochorous seeds, apart from the provision of a nutritive and attractive aril, is protection during their passage through the dispersal agent. This is not a critical feature of species where the aril is removed from the seed before ingestion (all myrmecochorous forms) but is important in those where the seed itself is swallowed (including nonarillate mimetic forms). Granivorous birds refused the red mimetic seeds of *Adenanthera* while fructivorous birds accepted them as berries, defecating them intact; and cockatoos failed to crack the very smooth, hard, and slippery seeds of *Abrus,* consequently accumulating a number in the rear of the beak (van der Pijl, 1982). It would, however, be facile to conclude that the hard testa of legume seeds evolved to

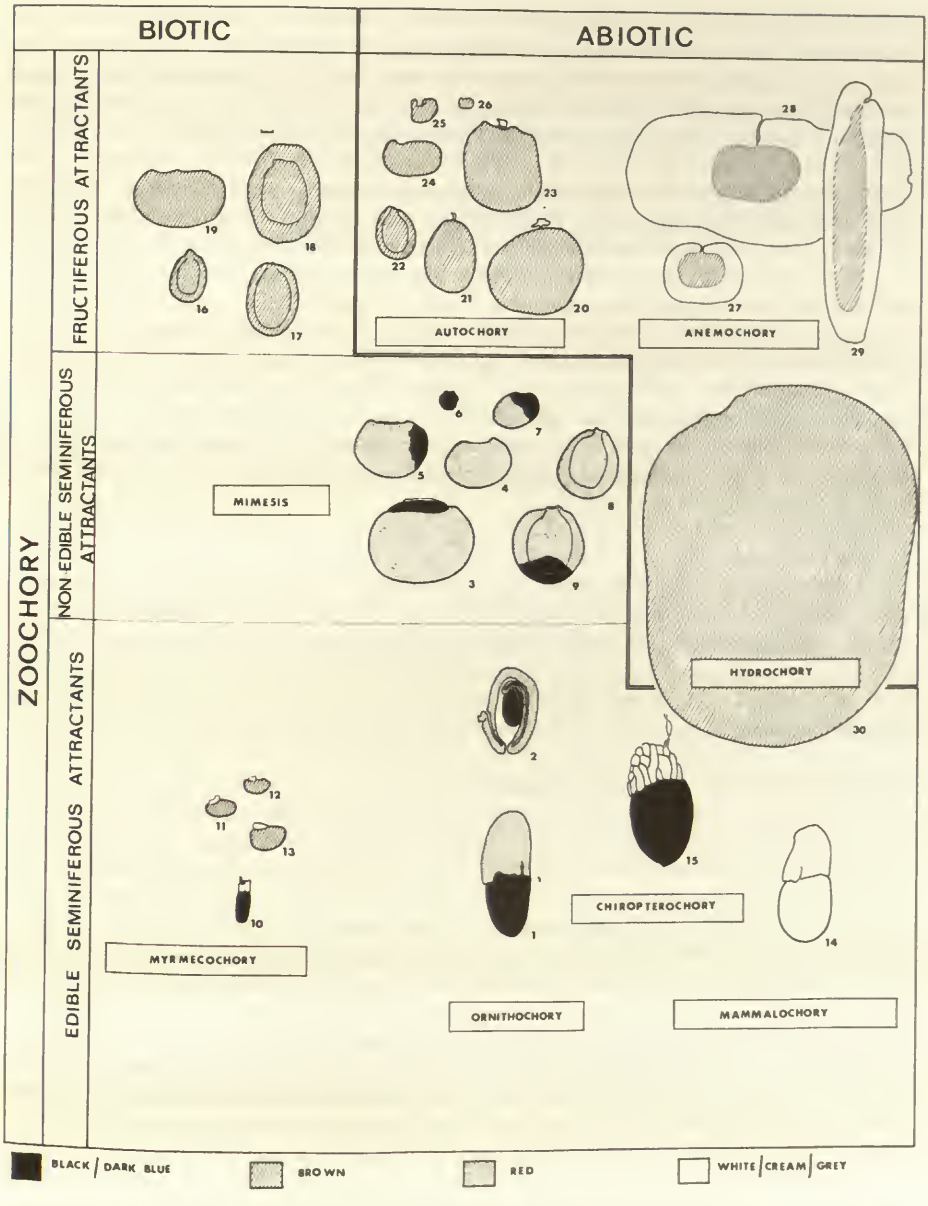

FIGURE 10. Adaptive radiation in strategies of seed dispersal in the Leguminosae. C Caesalpinioideae, M Mimosoideae, P Papilionoideae.—1. *Afzelia quanzensis* (C).—2. *Acacia cyclops* (M).—3. *Erythrina latissima* (P).—4. *E. lysistemon* (P).—5. *E. corallodendron* (P).—6. *Rhynchosia albiflora* (P).—7. *Abrus precatorius* (P).—8. *Adenanthara pavonina* (M).—9. *A. bicolour* (M).—10. *Acacia longifolia* (M).—11. *Liparia sphaerica* (P).—12.*Podalyria sericea* (P).—13. *Virgilia oroboides* (P).—14. *Schotia brachypetala* (C).—15. *Swartzia prouacensis* (P).—16. *Acacia tortilis* (M).—17. *A. nilotica* (M).—18. *Tamarindus indica* (C).—19. *Sophora japonica* (P).—20. *Schotia afra* (C).—21. *Caesalpinia decapetala* (C).—22. *Acacia farnesiana* (M).—23. *Milletia grandis* (P).—24. *Glycine wightii* (P).—25. *Crotalaria capensis* (P).—26. *Indigofera parviflora* (P).—27. *Indopiptadenia oudhensis* (M).—28. *Piptadeniastrum africana* (M).—29. *Newtonia aubrevillei* (M).—30. *Entada pursathea* (M).

protect them against digestion by birds or other animals. Nevertheless, passage through the gut may be beneficial. Passage of seeds of *Prosopis* through the gut of ruminants is well known to counterbalance the presence of an extremely hard testa (van der Pijl, 1982), and germination of undamaged seed of several species of *Acacia* is improved by passage through the digestive tract of gazelles (Halevy, 1974) and of *A. cyclops* by passage through the gut of birds (Glyphis et al., 1981). The testa of species of *Acacia* in which the indehiscent pods are consumed by herbivores and thus dispersed are therefore significantly thicker to prevent their being digested (Jackson & Moll, 1985). In such instances where germination is enhanced by consumption (e.g., *Acacia, Pithecellobium, Prosopis, Enterolobium, Cassia, Hymenaea, Dialium, Tamarindus*), germination may be considered to be delayed if the usual dispersal agent does not disperse the seed—the testa is hard enough to protect the seed during gut transit but then permeable to germination cues after defecation. Many legume seeds are rendered permeable after treatment with acid; thus it is not surprising that passage through an animal may result in improved germination. The structural effects are discussed elsewhere, but a clear site of action is on the lens. Whether the lens is a pre-adaptation to endozoochory can only be assessed after seed dispersal in the family has been more fully explored. However, the lens is also affected by fire and physical shock in some species. Significantly, Janzen et al. (1985) concluded that the adaptive significance of large mammals to *Enterolobium cyclocarpum* is in seed dispersal rather than in seed scarification.

Rather fewer legume genera have seeds specialized for other forms of dispersal, but some have the testa extended laterally as a chartaceous wing and are adapted for anemochory (many genera in the Mimosoideae) (Gunn, 1984), others for hydrochory or various unusual types of zoochory. In many others, the fruit wall assumes the role of reward, and attractants and various adaptations such as fleshy, edible pericarps or plume-like or burr-like pericarps are discussed by van der Pijl (1982).

SEED PREDATION

Morphological and Physiological Defenses

Seed predation may substantially reduce the effective reproductive output of a plant, and complete destruction of the seed crop by bruchid weevils is documented (Janzen, 1969, 1971). Beetles of the family Bruchidae are the most important seed predators in the Leguminosae (see Birch et al. and Johnson, this volume). Females oviposit on fruits or seeds, and the larvae feed inside a seed or seeds. These insects are for the most part, highly host-specific (Johnson, 1981a). Three predation guilds have been recognized (Johnson, 1981b): 1) mature pod guild—oviposit on seed pods only; 2) mature seed guild—oviposit only on mature seeds in pod or plant; 3) scattered seed guild—oviposit only on mature seeds on the ground. The coevolution between legumes and their bruchid predators is very close (Center & Johnson, 1974; Johnson, 1981a). Evolutionary responses of legumes to seed predation by these seed weevils are marked and include morphological and chemical features of the seed and fruit, as well as strategies of dispersal and fruiting phenology (Janzen, 1969, 1971; Johnson, 1977, 1981a, b). Those which relate to the seed itself include: 1) a layer of material on the seed surface that prevents entry of the larvae or swells when the pod opens and detaches the attached eggs; 2) a very dense and hard testa; 3) a hard and tough endosperm layer inside the testa that inhibits larval penetration; 4) immature seeds that remain very small throughout the year and then abruptly grow to maturity just before dispersal; 5) mature seeds so small that the ovipositing female cannot retain her balance on them; 6) seeds so thin that bruchids cannot mature in them; 7) more or less smooth or convex seed surfaces correlated with failure to oviposit by different bruchid species; 8) very moist and fleshy seeds that germinate within several weeks or die, thus bruchids lack time for development; 9) various chemical deterrents within the seed, including alkaloids, saponins, free amino acids, and endopeptidase inhibitors; 10) quantitatively or qualitatively inadequate food reserves (Janzen, 1981).

Virtually all of the many defensive traits seem effective against at least one species of bruchid (and the toxic and hallucinogenic chemicals probably also against other, usually mammalian or avian predators) but only rarely against all bruchids. In fact, most of the strategies are countered by one or a few species of bruchid, which consequently become specialist predators (Center & Johnson, 1974). Nearly all these defenses probably involve other functional systems in the plant relatively independent of direct bruchid attack (except perhaps some chemical defenses), and selection for the defensive properties of a trait will generally *not* proceed to the exclusion of other functions. The woody, hard-seeded Leguminosae in Central America (and probably elsewhere) that are attacked by Bruchidae can be distinguished by their smaller seeds, permitting the production of larger numbers of seeds per unit of photosynthetic ability; greater weight of seeds per unit of photosynthetic ability; and a reduction of chemical defensive compounds, which may in part make the first three strategies possible. Those species that are not attacked (through defenses of their own, usually seed toxins) are characterized by their larger seeds, smaller number of seeds, and lower weight of seeds per seed crop per unit of canopy (Janzen, 1969). The former strategy probably requires a decrease in seed size and the diversion of materials from the energy reserves of the plant that could have been used for vegetative growth or defense or for seeds of higher quality. The latter strategy is probably less taxing on the plant's energy budget but requires a more novel biochemical effort (Janzen, 1969).

Phenological Defense

An important component of the bruchid–legume interaction (and very likely that of any predator–seed interaction) is the escape component of dispersal. This is achieved by selection for phenology, diaspore morphology, and chemistry, etc., that maximizes seed removal by the dispersal agent most likely to place the seeds in the best sites in time and place for seedling survival. The importance of dispersal in avoiding predation can be assessed with the example of *Cassia grandis,* where most of the seed predation takes place before seed dispersal and there is apparently almost no postdispersal seed predation on the large hard seeds (Janzen, 1971). In fact, the structure and behavior of seeds and pods of many legumes are a result of combined selective pressure for seed dispersal and for protection from seed predators (Fig. 11). For example, small seeds probably pass through an animal's gut more easily than large seeds and may escape mastication. The unique winged fruits of *Nissolia* are wind-dispersed, which allows them to escape the depredations of their bruchid predator (Johnson, 1977), although it is difficult to assess the relative importance of colonization and predator escape in seed dispersal. Since the fruits are one-seeded, they may be regarded for these purposes as functionally equivalent to winged seeds. A spectacular morphobehavioral escape trait is that exhibited by *Arachis* spp., which have enormous seeds for their life-form but are likely quite free from predation by herbivores, and specifically bruchids, solely by virtue of the subterranean development of the fruits (Janzen, 1969).

It is only a short step from a seed predation that reduces the reproductive success of a plant to the predation of fleshy accessory tissues in which the seed escapes digestion and gains dispersal (Harper et al., 1970). Dispersal by vertebrates has a definite escape component: even if there is no insect attacking the seeds, escape from the dispersal agent and similar vertebrate predators must be selected. The simplest case of dispersal is where the seed is consumed with the fruit or carried in an intact ectochorous fruit. There should be strong selection for traits to withstand the chewing and digestive processes, such as a hard and acid-resistant testa. This may have led to selection for a testa hard enough to prevent damage to the embryo but soft enough that it is scarified, enabling the seed to germinate when defecated. Where the seeds are not destroyed by the dispersal agent, selection associated with dispersal should favor those dispersal agents that remove the seeds before seed-destroying animals get them and then carry the seeds to favorable habitats at the right time. A particularly interesting example of interaction between the dispersal agent in its beneficial role and

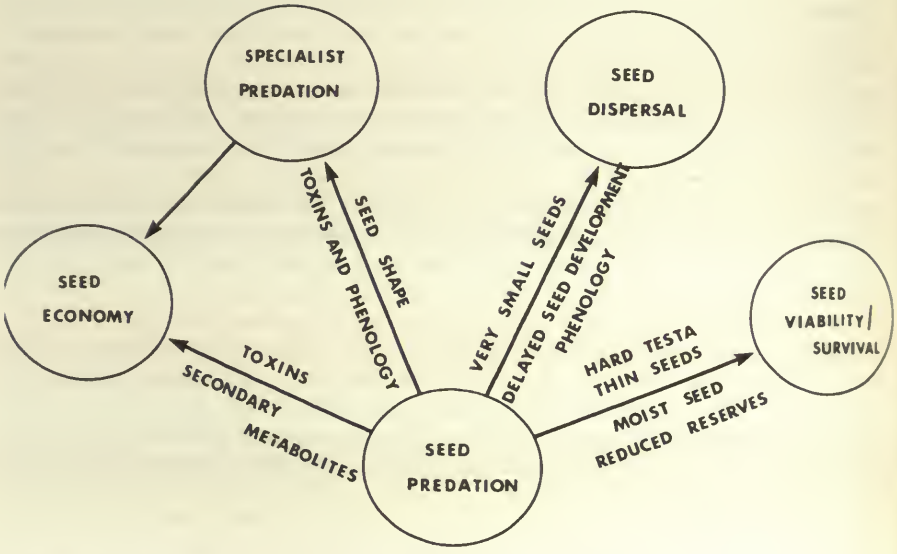

FIGURE 11. Mechanisms and constraints on predator avoidance.

as seed destroyer is associated with tropical or subtropical plants with black or otherwise dark seeds that are easily killed but also dispersed by birds. These plants have red or orange attractants external to the dark and essentially camouflaged seeds, suggesting that if the seeds are eaten, they are killed, but if they are dropped, they cannot be readily retrieved and thus survive (Janzen, 1969).

Seed Polymorphism

A final observation on color in predator avoidance is the role of color polymorphism in seeds, particularly common in the Papilionoideae. There is good evidence that most insects and mammals other than primates are more or less color-blind, so that color (as opposed to shade or pattern) is unlikely to influence predation. True trichromatic color vision, however, has been detected in practically all bird species investigated in this respect and seems to be normally present in lizards. Chromatic polymorphism of a prey species decreases the mortality due to (avian) predation, and a particular morph will have a three-fold selective advantage when occurring as one-third of a polymorphic population (Croze, 1970). This is due to a reduced reinforcement of a predator's search image. The importance of color polymorphism in legume seeds as a defense against predators has not been assessed but is confounded by the fact that color polymorphisms are usually linked to differences in germination behavior. However, in *Ononis sicula*, at least, it appears that the processes controlling color of the testa (from green to yellow, but ignoring a third brown morph not linked to this chromatic maturation series) are independent of those controlling the attainment of testa impermeability (Evenari et al., 1966).

Polymorphism in seed production in response to predation pressures is certainly ubiquitous within the Papilionoideae. Species of Ormosia in South America produce both red seeds and black and red seeds. The frequency distribution of these two seed morphs varies within and between species and between seasons on the same individual. Bicolored seeds are ornithochorous, highly toxic, and rarely attacked by bruchids, while the red ones are susceptible to bruchid predation (Stirton, pers. comm.). This suggests an advancement on the mere production of polymorphic seeds equivalent in

nutrient and energetic status: in these species of *Ormosia*, the black and red morphs are preferentially protected, and palatable red seeds appear to be produced in order to actively divert predation from them. This indicates that there are spatial and temporal variations in the intensity of bruchid attack and that the energetic costs of the two morphs are unequal. The physiological or nutrient status of the two morphs is unknown; nor is it known under what control the production of the morph is located, although the differences in annual response indicates one of great sensitivity. The phenomenon of "diversion seeds" has proceeded to a further stage in the South African species *Eriosema parviflorum*, in which the two seed morphs produced also differ in size and therefore probably nutrient and energetic status. *Eriosema parviflorum* produces reduced numbers of a large black seed morph and larger numbers of a smaller variegated black and brown morph. Field observations indicate that the variegated morphs alone are attacked by bruchids (Stirton, pers. comm.). Information on the relative frequencies of the morph, their relative susceptibility to predation (including the effect of the smaller size on bruchid maturation), camouflage potential, and their relative germinability are required. However, we may speculate that the energetic cost of the larger seeds must be greater than that of the smaller seeds and that the plant induces differential predation by producing larger numbers of a metabolically less expensive morph, probably of lower toxicity, nutrient content, and germinability.

DORMANCY

The Strategy

The biological purpose of a seed is conservation and dissemination of a species or species-lineage. To achieve this, it must germinate under conditions suitable for the maintenance of seedling growth and subsequent plant development, and dispersal is an important strategy in this campaign. Dispersal in time is an alternative to dispersal in space (Harper, 1982), and it may be a fitter strategy for seeds to remain dormant with or without spatial displacement. This will be so when the probability of a suitable environment (embracing biotic and abiotic influences) appearing respectively locally or temporally is greater than that of finding a suitable habitat at a distance or immediately.

Embryo Dormancy

During the period of dormancy, germination is prevented by various dormancy mechanisms that enable the seeds to germinate at the correct time and to survive adverse environmental conditions. Legume seeds can remain viable for extended periods of time (Hutton & Porter, 1937; Quinlivan, 1971). Germination of nearly all legume seeds is controlled by the impermeability of the testa and consequent inability of the seeds to imbibe water. With disruption of the testa and consequent hydration, germination proceeds rapidly. In some species, however, germination is inhibited even upon hydration, and dormancy is maintained by hormonal control. Innate, hormonally controlled dormancy is present in species with (*Cercis siliquastrum* [Profumo et al., 1979], *Trifolium* [Lipp & Ballard, 1959; Esashi & Leopold, 1969], *Medicago* and *Trigonella* [Lipp & Ballard, 1959], *Stylosanthes* [Burin et al., 1980]) and without (*Arachis* [Sreeramulu & Rao, 1969; Ketring & Morgan, 1969, 1970, 1971; Narasimhareddy & Swamy, 1979a, b]) a water-permeable testa. Van Staden et al. (1987) reviewed this literature.

Testa Impermeability

Impermeability of the testa occurs in all three subfamilies. Clearly it is associated with dehydration of the seeds during maturation, and the degree of drying of the seed is important in the

formation of impermeable seeds (Raleigh, 1930; Helgeson, 1932; Hutton & Porter, 1937; Stahl, 1937; Aitken, 1939; Zimmerman, 1942; Hurwitz & Gaurielth-Gelmond, 1952; Quinlivan, 1968).

In stored seeds of *Strophostyles helvola* impermeability of the testa is directly related to seed moisture content and increases rapidly after the moisture content falls below 21% (Hutton & Porter, 1937). The critical moisture content below which seeds become impermeable varies with the species, but is usually below 20% and sometimes (Lebedeff, 1943a) as low as 6%. The moisture content of the seeds is determined by the ambient relative humidity and the time of exposure (Aitken, 1939; Quinlivan, 1971). In *Phaseolus vulgaris* the percentage of hard seeds increases as the moisture content of the seeds decreases (Harrington, 1949). Drying these seeds over calcium chloride is effective in achieving this (Lebedeff, 1943a).

The relationship *relative humidity:seed moisture content:impermeability* has been noted in many species of the Papilionoideae (Hopkins et al., 1947; Nutile & Nutile, 1947; Hyde, 1954; Nakamura, 1962). The rate of softening of hard seeds increases as their initial moisture content increases (Lebedeff, 1943b; Gladstones, 1958; Quinlivan, 1968). Absolute and irreversible impermeability does develop, however, when the moisture content of the seeds falls below a critical percent (Quinlivan, 1968). The relationship *relative humidity* (more pertinently, the vapor pressure deficit or VPD):*seed moisture content* is an important one in an understanding of the development phase of testa impermeability, and it appears that this basic relationship is common to all or most papilionoids (Quinlivan, 1971). (In dormant species such as *Tipuana tipu*, which have the testa atrophied in response to persistent enclosure by a hard, indehiscent pod, it is probable that water movement into the seed is regulated by the pod wall.) The species do appear to differ, however, in the critical moisture contents at which absolute impermeability develops and in the degree of stability of semi-impermeable seeds (i.e., seeds that have a low water content but not yet below the critical percentage for induction of absolute impermeability) in a moist environment. Differences in impermeability within a species, however, in all probability reflect no more than differences in moisture content in the samples under test (Quinlivan, 1971). The length of the period of desiccation is as important as its severity. In *Trifolium subterraneum*, the longer the seeds are allowed to dry on the plant, the higher the percentage of hard seed (Aitken, 1939). In view of their higher moisture content, soft seeds of *T. subterraneum* are about 15% heavier than the hard, but on a dry weight basis are about 20% lighter than the hard. The higher dry matter content of the hard seed is probably due to the longer period of seed development on the plant, involving a higher nutrient concentration and consequently a high dry matter content (Aitken, 1939). The significance of seed size is discussed elsewhere.

Plants of *Ononis sicula*, a deserticolous legume that is facultatively a long-day annual, produce three types of seed, polymorphic in color, size, weight, and water permeability (Evenari et al., 1966; Gutterman & Evenari, 1972). In long days the mature seeds become yellow, relatively large and heavy, and are rather impermeable; in short days the mature seeds are green or brown, the former imbibing sooner than the yellow ones but with slight delay, and the latter imbibing immediately. When plants growing in long days die prematurely, seeds from pods that are immature at the time of death contain green or brown instead of yellow seeds, and these resemble closely in appearance and behavior those typical of plants growing in short-day conditions where seed development is always terminated before the yellow stage is reached. In all three kinds of seed, the embryos are ready to germinate; the difference in their germinability depends entirely on the permeability of their testa to water (Gutterman & Heydecker, 1973). The degree of impermeability is manifest in a corresponding difference in the degree of development of the cuticle (Gutterman & Heydecker, 1973) at least. It appears that long-day conditions permit the developmental processes of the seed to overtake those of the fruit, so that pods dehisce and seeds are shed when entirely mature. Short-day conditions, however, result in developmental processes of the seed lagging behind those of the fruit, so that pods dehisce and seeds are shed before maturation of the testa (coloration and permeability) (Evenari et al., 1966).

The Development of Testa Impermeability

Immature seeds, with their high moisture content, are larger than mature seeds of low moisture content. With the loss of water from the pod and enclosed seeds, the seeds gradually shrink in size. With this marked shrinking, the formerly pervious testa becomes impervious to water, providing the water loss takes place gradually and the shrinkage is consequently normal and uniform. Removal of the immature seeds of *Gymnocladus dioica* to a dry atmosphere or removal of the surrounding parenchymatous septae from the maturing pod invariably results in quick and uneven drying of the seeds, with consequent cracking and other abnormalities of the epidermis (Raleigh, 1930).

The path of moisture movement in and out of the papilionoid seed in response to changes in relative humidity was first elucidated by Hyde (1954) in the species *Trifolium repens*, *T. pratense*, and *Lupinus arboreus*. During seed ripening, the moisture content of the seed falls rapidly from over 150% to about 25%, and thereafter more slowly until the testa becomes impermeable at a moisture content of about 14%. Nearly 90% of the water lost from the seed during drying passes through the epidermal palisade and cuticle. When the moisture content falls to about 25%, however, the epidermis begins to resist the movement of water; and when the moisture content falls to about 14%, the epidermis becomes impermeable. These observations have been corroborated in other species (Quinlivan, 1968). In the upper part of each epidermis cell there are fluted thickenings of the radial walls which occupy most of the cell cavity (Hamly, 1932; Reeve, 1946; Hyde, 1954; Manning & Van Staden, 1985). As the seed shrinks during maturation, the strips of thickenings become pressed together until they entirely occlude the cell lumen and form an impervious barrier. The further drying and shrinkage of the seed that is made possible by the hilar mechanism increases the compression of the palisade layer and intensifies its impermeability (Hyde, 1954). The permeability of the epidermis being restricted at low moisture contents, the hilar counter palisade dies and shrinks, rupturing the epidermis along the median groove of the hilum. Thereafter, drying of the seed takes place increasingly by internal evaporation and diffusion of water vapor through the hilar fissure. When the moisture content is below 14%, drying takes place only in this way.

The hilar mechanism begins to function when the moisture content falls below 25%. The hilum acts as a hygroscopically controlled valve: when the ambient relative humidity is low, the hilar fissure opens, allowing the seed to lose moisture; when the relative humidity is higher outside the seed, however, the fissure closes, preventing the absorption of moisture. This results in impermeable seeds having a moisture content in equilibrium with the lowest relative humidity to which they have been exposed. The counter palisade is assumed to function as the motor tissue: when the ambient relative humidity falls below that of the seed interior, the counter palisade dries and shrinks, opening the fissure; when the relative humidity rises above that of the seed, the counter palisade swells and closes the fissure. In species lacking a counter palisade (*Erythrina* spp.) the hilar palisade presumably assumes this function (Manning & Van Staden, 1985).

The hilar valve does not function under conditions of very gradually increasing humidity, and presumably to close the hilum there must be a difference in moisture tension exceeding a certain minimum between the palisade epidermis and the counter palisade, which lie respectively inside and outside the impermeability barrier of the testa. Consequently, the intensity of dormancy is reduced greatly by storage of seeds under conditions of gradually increasing humidity (Hyde, 1954). During the early stages of ripening the hypodermal cells conduct the water passing from the center of the seed to the surface. As the seed ripens, they probably play a role in reducing excessive tangential stress in the drying palisade (which is essential in preserving the integrity of the impermeability of the palisade). During later stages of dehydration, the intercellular spaces of the hypodermis probably serve as a pathway for diffusion of water vapor to the hilum, and are continuous with those of the aerenchyma cells surrounding the tracheid bar (Hyde, 1954). The tracheid bar matures late in seed development (Manning & Van Staden, 1985) and probably functions as a central conduit where the moisture vapor is channeled during later stages of drying. Corner (1951)

suggested that it may hold water and maintain the turgidity of the developing seed under water stress, but its tardy development belies this.

Impermeability is a varietal characteristic, and it is possible to breed or select for a level appropriate to a particular environment or ecosystem. However, the structural and particularly chemical basis of varietal variability is not yet clear. Various anatomical features have been suggested to act as barriers to the passage of water. All are located in the outer epidermal palisade layer: a thick cuticle (White, 1908; Rees, 1911; Zimmerman, 1936; Khudairi, 1956); the suberization of the outer periclinal walls (Hamly, 1932; Aitken, 1939; Hyde, 1954; Spurny, 1964); hard pectic outer layers (Raleigh, 1930; Werker et al., 1973); the contraction of the palisade cells upon drying (Hyde, 1954; Corner, 1951); and the zone of the light line (Coe & Martin, 1920; Martin, 1922).

The cuticle is probably not a barrier to water movement (Aitken, 1939; Hyde, 1954), and it is now clear that the resistance to water movement is due mainly to deposition of a water-impermeable substance in the outer ends of the palisade cells. In most instances *(Melilotus, Trifolium, Lotus)* this has been recorded as suberin and/or cutin (Hamly, 1932; Watson, 1948; Hyde, 1954; Quinlivan, 1968), but in others *(Pisum, Gymnocladus, Trifolium subterraneum)* the suberized caps of the palisade have been embedded in a pectic cuticular matrix (Bhalla & Slattery, 1984).

The light line is an optical phenomenon generated by the juxtaposition of the inner cellulosic nature of the palisade cells and the outer suberized caps (Hamly, 1932; Aitken, 1939; Manning & Van Staden, 1985). The light line thus indicates the presence of an outer substance impermeable to water, and though the light line may be present at the lens before dehydration of the seed, its presence throughout is necessary for impermeability (Aitken, 1939). The functional difference between hard and soft seeds of *Trifolium subterraneum* has been ascribed to incomplete formation of the suberin caps in various regions of the testa of soft seeds as evinced by the absence of the light line (Aitken, 1939). However, the chemical and physical conditions that produce the light line (i.e., contraction of the cell walls and impregnation of the outer ends of the cells with suberin or pectin) are not alone responsible for palisade impermeability. It appears that in *Pisum*, phenolic compounds in the testa are oxidized by catechol oxidase during dehydration of the seed; the presence of these quinones in a continuous layer of either the palisade or osteosclereid cells (as occurs in hard-seeded species of *Pisum* only) is implicated in coat impermeability (Marbach & Mayer, 1974, 1975; Werker et al., 1979). Egley et al. (1985), however, found no trace of the oxidase enzyme during the development of the impermeable testa but instead found an increase in the peroxidase enzyme responsible for lignification. In permeable seeds the pectic caps are looser and quinones are discontinuous or absent in epidermal or hypodermal cells. The chemical and physical organization of the palisade layer of *Trifolium subterraneum* is identical in hard and soft seeds (Aitken, 1939; Slattery et al., 1982). Bhalla & Slattery (1984) found large amounts of callose in the parenchyma layers of impermeable but not permeable seeds and suggested that the presence of callose in this layer controls the permeability in clover. In *Cercis*, the major reason for impermeability is a lipidic noncellular layer situated between the inner surface of the testa and the endosperm; imbibition of endosperm and embryo is possible only when this layer is damaged (Riggio-Bevilacqua et al., 1984; 1985). In this species, dormancy is largely under the control of the copious inhibitory endosperm.

Because seeds of the Caesalpinioideae and Mimosoideae lack the complex hilum of the Papilionoideae, regulation of desiccation is not under hilar control. However, a few caesalpinioid seeds and several mimosoid seeds have a pleurogram. Opening and closing of the pleurogram due to changes in moisture content outside the seed permit desiccation of the seed in an analogous, although more rudimentary, manner. Seeds without a pleurogram presumably rely on water loss through the complete testa to reduce the moisture content to a level low enough to induce impermeability. In such seeds, drying is presumably hampered by fluctuations in the ambient humidity, since moisture entry into the seed may not be impaired (but see note on *Cercis* above) until the seed has been subjected to conditions of sufficiently low humidity to induce impermeability. Consequently, nonpapilionoid species lacking a pleurogram may be expected to occur in areas with a rather dry period during seed maturation. It is possible that the characteristically smaller seeds of

many of the Mimosoideae dry more rapidly and completely than the larger seeds common in the Caesalpinioideae. In *Acacia*, however, more moisture is lost when the lens remains uncovered than when it is covered, and it possibly serves as an outlet to the environment until the moisture content of the seed is low enough to render it impermeable. Seeds of *Bauhinia* lack a pleurogram, but most of the surface (hilar region excepted) bears sunken stomata (Bragg & Bridges, 1984), the function of which is unknown. Pleurograms are known only in the Leguminosae and Cucurbitaceae.

BREAKING OF DORMANCY

Mechanisms of Breaking Dormancy

Seeds of *Arachis* and *Stylosanthes* require a period of after-ripening to overcome embryo dormancy, when levels of abscisic acid (ABA) in the embryo decrease and levels of cytokinins increase. The role of cytokinins in the seeds appears to include counteracting ABA; breaking dormancy, probably by stimulating ethylene production; and stimulating respiration and reserve mobilization in the cotyledons. The endosperm in *Cercis* inhibits embryo growth, and a period of stratification is necessary to effect the physiological changes required to permit embryo growth. Embryonic dormancy may be considered a method whereby germination is delayed until appropriate environmental cues occur. In this way germination at ecologically inappropriate times is forestalled even though the testa may have been rendered permeable prior to this. Artificial breaking of dormancy in impermeable legume seeds has relied on methods for rendering the testa permeable. This has been achieved through various methods (Porter, 1949; Quinlivan, 1971), but all treatments that have proved successful can be classified into one of three categories: 1) chemical weakening of the testa; 2) induced stresses on testa structure; or 3) gradual increase in seed moisture content. Chemical scarification using concentrated acids, usually sulfuric, is a well-recognized method of softening impermeable testa and is successful in many species. Alcohol has proved effective in a few cases (Cavazza, 1951), particularly among the Caesalpinioideae (Barton, 1947). Stresses on the testa can be induced by: temperature fluctuations, e.g., freezing, heating with dry or more commonly, wet heat, or alternating heating and cooling; impaction; pressure; or radioactive treatments. In many papilionoid legumes two types of impermeability occur in the seed crops. Semi-impermeable seeds have a higher moisture content (above 10% dry weight in *Lupinus varius*) (Quinlivan, 1968) and soften slowly when placed in a humid atmosphere or moist soil, the rate of softening varying directly with the moisture content. Moisture penetration occurs at random sites over the testa, but not normally at the hilum or lens. In absolutely impermeable seeds the moisture content is low (less than 8.5% dry weight in *L. varius*), and they do not soften when placed in a humid atmosphere. Such seeds require treatments 1 or 2 to break the impermeability. These treatments result in fracturing of the testa at the lens, through which water penetration subsequently occurs.

The Ecology of Dormancy Breaking

Natural softening of legume seeds is discussed by Aitken (1939), Quinlivan (1968, 1971), and Cavanagh (1980). Natural chemical scarification can occur through the action of microbial agents, digestive juices of animals, or organic acids. Temperature fluctuations occur through climatic and often environmental (e.g., fire) factors. In *Lupinus varius* grown in Australia, for instance, the autumn germination of the species depends on how desiccated the seeds become during the summer. Seeds with a moisture content above 10% are semipermeable and soften at a rate dependent directly on their initial moisture content. This rate is independent of temperature fluctuations and results in scattered germination over several months. Seeds that have dried to moisture contents below the

critical level (ca. 8.5%) soften only through fracturing of the testa at the lens, caused by fluctuating temperature. Seeds softened in this way germinate immediately after the soil has been wetted. Much of southern Australia has a hot, dry summer environment which would dry seeds below the critical level and bring about a cumulative softening by cracking of the testa at the lens through diurnal temperature fluctuations. Once the lens is cracked the condition is irreversible (Hamly, 1932), although apparently reversible in some other species upon further dehydration (Aitken, 1939; Hagon & Ballard, 1970). Drought following initial moisture would result in loss of seed viability in species with an irreparable breach in the lens. A qualitative softening would thus be valuable. Particularly along the south coast of Australia, relative humidity during the summer is too high to cause sufficient moisture loss from some seeds. In such regions the pattern of autumn germination would be from low moisture-content hard seeds fractured at the lens, followed by a scattered germination from higher moisture-content semipermeable seeds. A two-fold pattern of germination of this type has much to recommend it, particularly in regions of unreliable rainfall (Quinlivan, 1971).

It is not possible to assess the relative importance of the two degrees of impermeability or the two types of softening mechanisms. In arid areas with hot, dry summers the absolutely impermeable seeds are probably in the majority. In other environments (including the strictly Mediterranean), where seeds form during a relatively cool, moist period, most seedling recruitment is probably due to softening of semi-impermeable seeds of high moisture content. However, the two distinct phases of the impermeability mechanism probably have played a part in allowing different species of the Papilionoideae to adapt successfully to a wide range of environments.

Polymorphism in germination characteristics within a seed crop is not uncommon in the Papilionoideae (Evenari et al., 1966; Gutterman & Evanari, 1972; Saha & Takahashi, 1981; Kelly & Van Staden, 1985), but its occurrence in the Caesalpinioideae or Mimosoideae is doubtful based on current knowledge. Seed impermeability characteristics are heritable (Lebedeff, 1943b), but are induced environmentally in many cases (Evenari et al., 1966). The relationship between color and physiology is usually marked, but the functional significance of this is not yet clear. Polymorphism in color may reflect differences in chemical changes (e.g., oxidation of phenols) due to incomplete drying, or may reflect the influence of completely different ecological pressures.

The three seed color morphs produced by the deserticolous annual *Ononis sicula* (see above), differ also in their germination characteristics. Aside from the brown seeds, it seems that as the testa matures, it changes from green to yellow (Evenari et al., 1966). A change of day length during the last eight days of ripening can affect the color, size, weight, water permeability, and germination of seeds. The vast year-to-year differences in occurrence of *O. sicula* in the wild are thus determined in part by the environment to which the mother plant is exposed (i.e., the season in which it has grown, and therefore the time available to plant growth); and by the degree of desiccation after seed dispersal (Gutterman & Evenari, 1972).

Often, seeds that are slow to germinate have thicker and less permeable testa (Noodén et al., 1985). Environmental factors can influence testa development, apparently through effects on the maternal plant, and hormone treatments of the parent plant may alter testa thickness and permeability of the seeds produced (Van Staden et al., 1986). Cytokinin and mineral nutrients moving through the xylem can control testa development in soybean, and lack of these substances results in the development of a thicker, less permeable testa (Noodén et al., 1985). The availability of minerals and hormones is affected by stress conditions experienced by the maternal plant.

The Role of the Lens

The quality of the impermeability (i.e., softening under moist conditions as compared to absolute impermeability) is controlled by the suberization of the epidermis and the tension of the lens region, where the palisade cells are elongated and bent and under a natural condition of stress. Soft seeds differ from hard ones in the presence of a split between the palisade cells at the lens or in permeable

patches in the rest of the testa (Aitken, 1939). The lack of impermeability in *Vigna umbellata* is associated with the presence of parenchymatous tissue beneath the lens (Gopinathan & Babu, 1985), and the epidermis is apparently disrupted at the lens; hard-seeded species of *Vigna* have sclerenchymatous tissue beneath the lens and an apparently intact epidermis. Hard seeds of legumes become permeable through the propensity of the lens palisade to split under pressure (Hamly, 1932; Aitken, 1939; Hagon & Ballard, 1970). This results from exposure to any of the stress-inducing factors mentioned, and the lens also is very susceptible to attack by chemical scarification. Water entry in treated seeds is solely through the lens. The lens is thus the limiting inbuilt point of weakness in absolutely hard seeds, but the lens and testa should be regarded as an integrated system. The mechanical properties of the remainder of the testa (as opposed to its water impermeability) are important in transmitting stresses to the lens, and rupturing of the palisade at the lens can be induced by percussion on other areas of the seed (Ballard, 1976). Furthermore, the seeds of certain legumes are susceptible to cellular rupture during imbibition when the testa is damaged or missing, and the testa of these legume seeds acts as protection against rupture of embryo cells during imbibition (Duke & Kakefuda, 1981).

In the Mimosoideae the lens is similarly implicated as the seed structure on which the majority of treatments, especially heat, have their effect in breaking seed hardness (Cavanagh, 1980). The opening of the lens in *Acacia* is induced by heat, and is effected through the splitting of thin-walled cells lying beneath short palisade cells within the lens, and in *Albizia* is presumably related similarly to mechanical weakness of the associated hypodermis (Dell, 1980). Estimated pressure increases of greater than 25% within the vascular bundle, which passes directly under the thin-walled cells, might influence lifting of the lens (Hanna, 1984). The vascular bundle between the hilum and adjacent lens is virtually devoid of xylem vessels, thereby inhibiting water entry through the hilum, but following the lens the vascular bundle is enlarged and facilitates passage of water, and may influence lifting of the lens. In *Albizia* the effect of heat may be more dramatic, resulting in lifting and ejection of the lens plug. After the lens is lifted, water enters via the lens cavity and passes into the testa and through the parenchyma to the embryo. Movement of water through the testa is facilitated by the vascular bundle, which arches close to the lens, and a sharp front of water runs toward the chalazal end of the seed, corresponding with unequal swelling of the seed (Dell, 1980). This may explain the reduced or absent post-chalazal extension of the vascular bundle in the Papilionoideae: the curvature of the embryo brings the chalaza near the site of water entry at the lens, facilitating even swelling of the embryo without requiring directed water transport to the chalazal end of the embryo.

Hard-seeded species of *Acacia (A. farnesiana, A. kempeana, A. tenuissima,* and *A. wiseana)* have a well-developed lens and associated sublens parenchyma. Heat treatment, among others, causes a break in these cells and lifting of the lens. In contrast, soft-seeded species *(A. cambagei)* have a poorly developed lens, and heat treatment does not cause rupture of the lens.

Clearly, the functional attributes of the lens are analagous in all impermeable legume seeds, although anatomical differences between genera and subfamilies influence the effect of various treatments and the operation of the lens. The irreversibility of lens rupture seems invariable in mimosoid seeds, but not always in papilionoid seeds, and the role of the vascular bundle passing beneath the lens is not clear in post-developmental processes. The ecological significance of the difference in lens functioning is not in doubt, but has been studied little (see Cavanagh, 1980, for comments on *Acacia*). The structural variation evinced by the lens within the Papilionoideae (Zimmerman, 1936) testifies to its adaptive significance and sensitivity to ecological selection, and to its contribution to the evolutionary success of the subfamily. In Mediterranean plant communities, seed germination can be important in pyric regeneration (Naveh, 1975); the cyclic occurrence of various understory or shrubby leguminous species in Australia and South Africa following fires is probably due to the seeds having a thick, heat-resistant testa and a heat-sensitive lens.

Thus an evolutionary progression is conceivable, beginning in the moist tropics with a caesal-

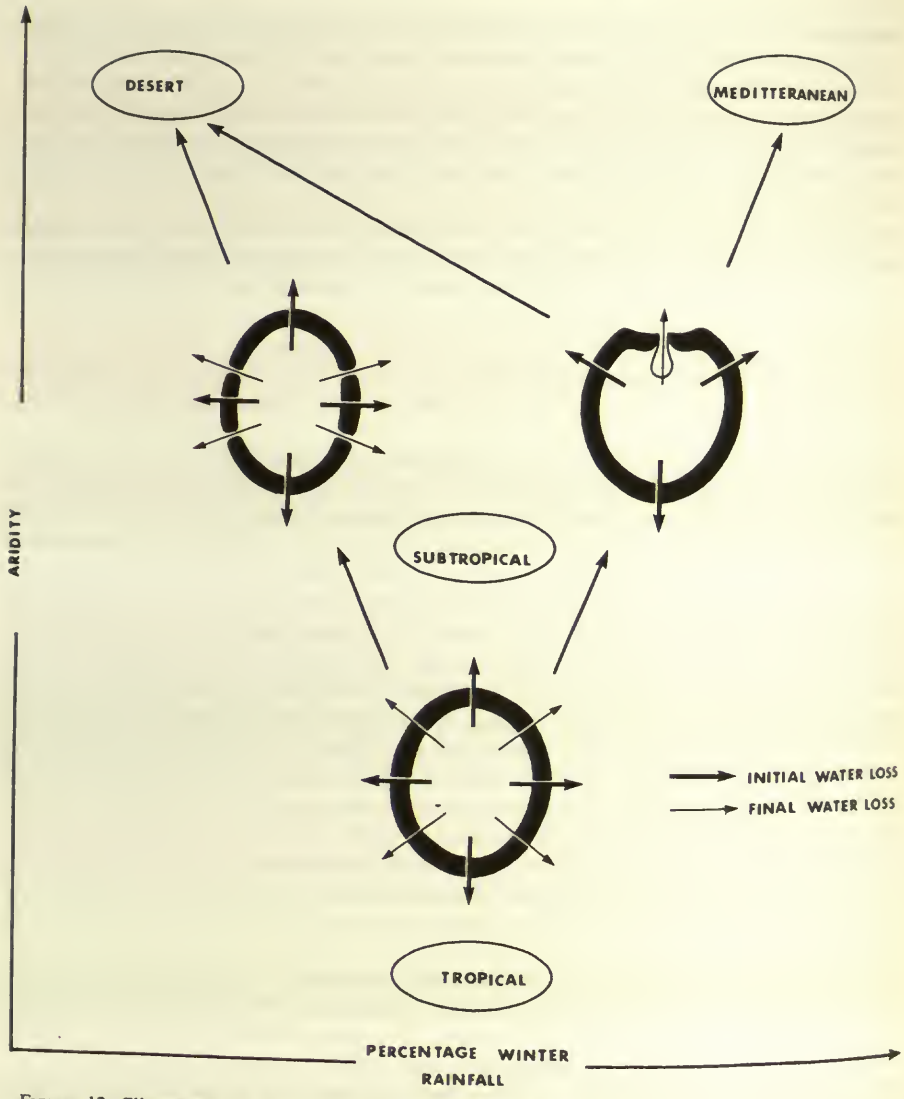

FIGURE 12. Climate and the possible evolution of the legume seed.

pinioid ancestor having pleurogramless seeds that lose moisture across the entire testa surface (Fig. 12). Next we may have some subtropical, followed by Mediterranean, species having a rudimentary valve in the form of a pleurogram (which permits continuous drying) and a temperature-sensitive lens.

In another line that lacked a pleurogram, the hilar region assumed the function of a valve. This permitted precisely controlled desiccation and a dimorphic/polymorphic dormancy suited to exploitation of very arid seasonal and moist temperate areas alike. Embryo dormancy is a further strategy for controlling germination until environmental cues are experienced, which normally presage conditions favorable for seedling survival. In this way germination at ecologically inappropriate periods is limited, even though the testa may be permeable.

IMBIBITION AND GERMINATION

The Testa

If a seed is sown onto the surface of a water-supplying substrate, it will germinate only if it absorbs water from that substrate more rapidly than it loses it to the atmosphere. For this to be so: the seed must make good contact with the available water; the tension on the water must be relatively low; or the surrounding atmosphere must be moist (Harper & Benton, 1966). The wide variation in emergence that occurs when the same seed lots are sown at different sites (Heydecker, 1969) has demonstrated that the emergence of peas is very sensitive to the soil environment. Germination of many species is markedly influenced by the tension of the available water. Mucilaginous seeds show least sensitivity to tension (because of a large surface contact and a reduced water loss to the atmosphere from the hydrophilic mucilage), while spiny and reticulate seeds are particularly sensitive (Harper & Benton, 1966). The cuticle of some legumes is hydrophilic, becoming gelatinous when wet (*Caesalpinia decapetala* and other caesalpinioid and mimosoid genera with thick cuticles) or the parenchymatous layers may swell on hydration *(Schotia, Delonix),* but most legume seeds have a testa that is smooth and hard at low magnifications even when wet. The exceptional species or genus has tuberculate seeds (*Glycine* spp., *Ononis* spp., *Lupinus hirsutus, Melilotus indica*), although the epitesta of some seeds (*Glycine* spp.) may be functionally analogous to a sculptured or mucilaginous testa. At high magnification, however, the smooth testae appear to be finely patterned. Such microsculpturing is unlikely to be important in influencing uptake of water, although it may influence oviposition by bruchids (Janzen, 1981). Nevertheless, subtle differences in seed shape and surface interacting with subtle variations in the structure of the soil surface can be important in influencing the abundance of particular species and the balance between species (Harper et al., 1965).

The lens is the site of water entry in seeds that have been rendered permeable. The importance of the lens appears to be dependent upon its structure and its position; the site and rate of water entry has an effect on seedling vigor (Manning & Van Staden, 1987b). Disruption of the lens in *Sesbania punicea* is more effective in stimulating germination, and results in greater seedling vigor, than complete excision of this region. However, the rate of germination in seeds where the lens has been excised completely is enhanced greatly when the availability of water is controlled by an inert osmotica. Water entry through the disrupted lens probably is controlled by the hypodermal sclereids, which have thick secondary walls of loose and permeable structure (Manning & Van Staden, 1987b).

The Endosperm

A number of legumes have endospermous seeds, and generally these seeds contain galactomannan polysaccharides localized in the cell walls of the endosperm. These galactomannans usually are ascribed a storage role analagous to that of the starch in the cotyledons of many exendospermous legume seeds. However, the galactomannan reserves in the endosperm of *Trigonella foenum-graecum* are not necessary for the germination of the embryo, and there is no evidence of a nutritional function quantitatively different from that of the embryo reserves (mainly lipid and protein) (Reid & Bewley, 1979). The endosperm is a key tissue in the interaction of the seed with water during imbibition and up to the completion of germination. The endosperm takes up over 60% of the water imbibed by the hydrating seed and is able to buffer the germinating embryo against desiccation if the imbibed seed is subjected to drought-stress (Reid & Bewley, 1979), a role which could not be mimicked by lipid, storage protein or starch. The endosperm thus fulfills two physiological functions in succession.

The Embryo

The curvature of the embryonic axis in papilionoid seeds may result in greater protection of the radicle (Gunn, 1984). It also brings more of the embryo closer to the lens, which is the site of water entry. This conceivably results in more efficient and even swelling of the embryo, with less damage due to uneven swelling. Papilionoid seeds almost invariably imbibe more rapidly than caesalpinioid or mimosoid seeds of comparable size.

Characteristically during seed germination, the principle reserve materials in the cotyledons or endosperm are degraded and the products arising from the reserve tissue are translocated to the growing seedling. The utilization of the reserve components and normal development of the reserve tissues are dependent upon the presence of an attached embryo axis. Endosperm tissues or cotyledons from which the embryo axes have been removed fail to show the characteristic loss of reserve components (Van Staden et al., 1986). The control of reserve hydrolysis and mobilization by the axis is via the production of hormones, particularly cytokinins, which are apparently transported to the cotyledons where they initiate activity of hydrolytic enzymes. It is likely that at least some of the cytokinins are synthesized in the embryonic radicle. The large papilionoid radicle in extensive contact with cotyledon tissue may be more efficient in stimulating reserve mobilization.

GERMINATION AND ESTABLISHMENT

Seed size influences germination, emergence, early vegetative growth, and in herbage legumes at least, producitivity (Black, 1959). Seed size in legumes is constrained by successional status, dispersal strategy, seed predation, and ecological aspects of seed germination and seedling establishment. These selective forces acting in combination will determine the seed size phylogenetically most appropriate for the species. Selection for increased seed size in legumes is successful in as little as three cycles of selection (Draper & Wilsie, 1965; Fehr & Weber, 1968), and gains of 20% per cycle have been achieved in *Lotus corniculatus* (Draper & Wilsie, 1965). There has been some success in selecting for protein and oil content in *Glycine max,* with a heritability of 0.93 for seed size, 0.87 for specific gravity, 0.77 for protein, and 0.73 for oil (Fehr & Weber, 1968). Maximum success for high protein and low oil content was achieved by selecting for large seed and high specific gravity; and for low protein and high oil by selecting for small seed and low specific gravity. Seedlings from larger seeds show greater seedling vigor (Black, 1956; Twamley, 1967), and in *Trifolium subterraneum* this appears to be due to the greater area of the cotyledons rather than to their weight and reserve food supply (Black, 1956, 1957a). Increasing seed weight resulted in increased seedling dry weight and increased leaf area, although the relative growth rate of seedlings of *Vicia dasycarpa* is unaffected (Williams, 1967). In general the leaf weight and leaf area of *Trifolium subterraneum* is markedly dependent on embryo (seed minus testa) weight (Black, 1957a). However, the initial advantage in growth possessed by large seeds ceases to be significant when the leaf area index (leaf area to ground area) of the seedling approximates the value of four (Black, 1957b). At this stage the limiting factor affecting rapidity of growth becomes the amount of light reaching the photosynthetic tissues.

For successful germination, imbibition of water from the substrate by the seed must exceed loss to the atmosphere. The problem of desiccation is not as acute for small seeds as it is for large ones because small seeds have a larger contact/surface area ratio. This suggests that large-seeded species may be greatly disadvantaged in open habitats where they are more prone to desiccation. Small seeds, however, are less susceptible to desiccation and more likely to be able to germinate in open habitats (Harper & Benton, 1966). A seed with a small supply of reserve material must germinate and establish rapidly during favorable periods if the seedling is to survive the onset of adverse conditions. The microclimatic changes in a closed habitat are less severe, and slow-growing seedlings from large seeds are at less disadvantage in that environment (Sheldon, 1974). This indicates

the strategic differences between a small-seeded annual species inhabiting open and disturbed ground and a larger-seeded perennial living in closed communities. Considering the ecological conditions, the typically smaller seeds of the Papilionoideae (Gunn, 1984) are likely to have been important in the exploitation of drier and more seasonal climes by this subfamily. Seed size and form is not determined by any single selective influence but is a manifestation of all selective forces to which a plant is subject.

FINAL COMMENT

Seed survival is important only if successful germination and growth, resulting in seed production for the following generation, occurs also. Selection is thus as varied and as intense on the seed as it is on the plant. Although by its nature the seed may be constrained to offer more passive solutions, it is not less active in promoting its success. The seed should not be considered as a mere ancillary to plant survival, but as a highly integrated aspect of environmental exploitation. In fact, in certain species the seed stage is as vital to the genotypic perpetuation of the species as the flowering stage. The seed must obtain nutrients, dissemination agents, appropriate requisites for imbibition and germination, and avoid predation in ways that enhance its chances of success in all these areas. However, the importance of chance events in launching a particular strategy must not be overlooked. The relative advantages of a strategy may be rather small. Once a species (or other taxon) has been started on a strategy, further evolution may be more or less canalized as options become limited or less feasible, through genetic canalization or environmental and biotic pressures. The result is that unique compromise most successful in ensuring the transmission of the genotype through time and space.

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REFERENCES

AITKEN, Y. 1939. The problem of hard seeds in subterranean clover. Proc. Roy. Soc. Victoria 51: 187–213.

BALLARD, L. A. T. 1976. Strophiolar water conduction in seeds of the Trifoleae induced by action on the testa at non-strophiolar sites. Austral. J. Pl. Physiol. 3: 465–469.

BARTON, L. V. 1947. Special studies on seed coat impermeability. Contr. Boyce Thompson Inst. Pl. Res. 14: 355–362.

———. 1961. Experimental seed physiology at Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, U.S.A. 1924–1961. Proc. Int. Seed Testing Assoc. 26: 561–596.

BERG, R. Y. 1975. Myrmecochorous plants in Australia and their dispersal by ants. Austral. J. Bot. 23: 475–508.

BHALLA, P. L. & H. D. SLATTERY. 1984. Callose deposits make clover seeds impermeable to water. Ann. Bot. 53: 125–128.

BLACK, J. N. 1956. The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterraneum* L.). Austral. J. Agric. Res. 7: 98–109.

———. 1957a. The early vegetative growth of three strains of subterranean clover (*Trifolium subterraneum* L.) in relation to size of seed. Austral. J. Agric. Res. 8: 1–14.

———. 1957b. Seed size as a factor in the growth of subterranean clover (*Trifolium subterraneum* L.) under spaced and sward conditions. Austral. J. Agric. Res. 8: 335–351.

———. 1959. Seed size in herbage legumes. Herbage Abstr. 29: 235–241.

Bragg, L. H. & T. L. Bridges. 1984. Testa characterization of selected Caesalpinioideae (Leguminosae) genera. Scanning Electron Microscopy. 4: 1751–1758.

Bresinsky, A. 1963. Bau, Entwicklungsgeschichte und Inhaltsstoffe der Elaiosomen. Studien zur myrmekochoren Verbreitung von Samen und Früchten. Biblioth. Bot. 126: 1–54.

Burin, M. E., R. S. Barros & A. B. Rena. 1980. Chemical regulation of endogenous dormancy of the seeds of *Stylosanthes humilis* H.B.K. Pl. Physiol. 65 (Suppl.): 42.

Cavanagh, A. K. 1980. A review of some aspects of the germination of Acacias. Proc. Roy. Soc. Victoria 91: 161–180.

Cavazza, L. 1951. The effect of alcohol on hard seeds. Nuovo giornale botanico italiano e Bolletino della Società botanica italiana 58: 393–397.

Center, T. D. & C. D. Johnson. 1974. Coevolution of some seed beetles (Coleoptera: .Bruchidae) and their hosts. Ecology. 55: 1096–1103.

Coe, H. S. & J. N. Martin. 1920. Sweet-clover seed. U.S.D.A. Bull. No. 844: 1–39.

Corner E. J. H. 1951. The leguminous seed. Phytomorphology 1: 117–150.

———. 1976. The Seeds of Dicotyledons, Vols. 1 & 2. Pp. 311, 552. Cambridge Univ. Press, Cambridge.

Croze, H. 1970. Searching image in carrion crows. Parey, Berlin.

Dell, B. 1980. Structure and function of the strophiolar plug in seeds of *Albizia lophantha*. Amer. J. Bot. 67: 556–563.

Draper, A. D. & C. P. Wilsie 1965. Recurrent selection for seed size in birdsfoot trefoil, *Lotus corniculatus* L. Crop Sci. 5: 313–315.

Duke, S. H. & G. Kakefuda. 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. Pl. Physiol. 67: 449–456.

Egley, G. H., R. N. Paul, S. O. Duke & K. C. Vaughn. 1985. Peroxidase involvement in lignification in water-impermeable seed coats of weedy leguminous and malvaceous species. Plant, Cell and Environ. 8: 253–260.

Esashi, Y. & A. C. Leopold. 1969. Dormancy regulation in subterranean clover seeds by ethylene. Pl. Phsyiol. 44: 1470–1472.

Evenari, M., D. Koller & Y. Gutterman. 1966. Effects of the environment of the mother plant on germination by control of seed coat permeability to water in *Ononis sicula* Guss. Austral. J. Biol. Sci. 19: 1007–1016.

Fehr, W. R. & C. R. Weber. 1968. Mass selection for seed size and specific gravity in soybean population. Crop Sci. 8: 551–554.

Gladstones, J. S. 1958. The influence of temperature and humidity in storage on seed viability and hard-seededness in the West Australian blue lupin, *Lupinus digitatus* Forsk. Austral. J. Agric. Res. 18: 713–731.

Glyphis, J. P., S. J. Milton & W. R. Siegfried. 1981. Dispersal of *Acacia cyclops* by birds. Oecologia. 48: 138–141.

Gopinathan, M. C. & C. R. Babu. 1985. Structural diversity and its adaptive significance in seeds of *Vigna minima* (Roxb.) Ohwi & Ohashi and its allies (Leguminosae-Papilionoideae). Ann. Bot. 56: 723–732.

Gunn, C. R. 1981a. Seed topography in the Fabaceae. Seed Sci. and Technol. 9: 737–757.

———. 1981b. Seeds of Leguminosae. Pp. 913–925 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———. 1984. Fruits and seeds of genera in the subfamily Mimosoideae (Fabaceae). Tech. Bull. U.S.D.A. Number 1681.

Gutterman, Y. & M. Evenari. 1972. The influence of day length on seed coat colour, an index of water permeability, of the desert annual *Ononis sicula* Guss. J. Ecol. 60: 713–719.

———& W. Heydecker. 1973. Studies on the surfaces of desert plant seeds. 1. Effects of day length upon maturation of the seed coat of *Ononis sicula* Guss. Ann. Bot. 37: 1049–1050.

Hagon, M. W. & L. A. T. Ballard. 1970. Reversibility of strophiolar permeability to water in seeds of subterranean clover *(Trifolium subterraneum)*. Austral J. Biol. Sci. 23: 519–528.

Halevy, G. 1974. Effects of gazelles and seed beetles (Bruchidae) on germination and establishment of *Acacia* species. Israel J. Bot. 23: 120–126.

Hamly, D. H. 1932. Softening of the seeds of *Melilotus alba*. Bot. Gaz. 93: 345–375.

Hanna, P. J. 1984. Anatomical features of the seed coat of *Acacia kempeana* (Mueller) which relate to increased germination rate induced by heat treatment. New Phytol. 96: 23–29.

HARDHAM, A. R. 1976. Structural aspects of the pathways of nutrient flow to the developing embryo and cotyledons of *Pisum sativum* L. Austral. J. Bot. 24: 711–721.

HARPER, J. L. 1982. Population Biology of Plants. Academic Press, London.

_____ & R. A. BENTON. 1966. The behaviour of seeds in soil II. The germination of seeds on the surface of a water supplying substrate. J. Ecol. 54: 151–166.

_____, P. H. LOVELL & K. G. MOORE. 1970. The shapes and sizes of seeds. Annual Rev. Ecol. Syst. 1: 327–357.

_____, J. T. WILLIAMS & G. R. SAGAR. 1965. The behaviour of seeds on soil I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. J. Ecol. 53: 273–286.

HARRINGTON, J. F. 1949. Hard seeds in beans and other legumes. Seed World 64: 42, 44.

HEDLEY, C. L. & M. J. AMBROSE. 1980. An analysis of seed development in *Pisum sativum* L. Ann. Bot. (London) 46: 89–105.

HELGESON, E. A. 1932. Impermeability in mature and immature sweet clover seeds as effected by conditions of storage. Trans. Wisconsin Acad. Sci. 27: 193–206.

HEYDECKER, W. 1969. Report of the Vigour Test Committee, 1965–1968. Proc. Int. Seed Testing Assoc. 34: 751–773.

HOCKING, P. J. & J. S. PATE. 1977. Mobilization of minerals to developing seeds of legumes. Ann. Bot. (London) 41: 1259–1278.

HOPKINS, E. F., F. J. S. RAMIREZ, V. PAGAN & A. G. VILLAFANE. 1947. Investigations on the storage and preservation of seed in Puerto Rico. Puerto Rico Agric. Exp. Sta. Bull. 72.

HURWITZ, S. & H. GAURIELTH-GELMOND. 1952. Hardening of sweet lupin seeds. Klavim 2–3: 57–64.

HUTTON, M. E. & R. H. PORTER. 1937. Seed impermeability and viability of native and introduced species of Leguminosae. Iowa State Coll. J. Sci. 12: 5–24.

HYDE, E. O. C. 1954. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. Ann. Bot. (N.S.) 18: 241–256.

ISELY, D. 1955. Observations on seeds of the Leguminosae: Mimosoideae and Caesalpinioideae. Proc. Iowa Acad. Sci. 62: 142–145.

JACKSON, P. & E. MOLL. 1985. Do plants love animals that eat them? Veld & Flora 71: 26–27.

JANZEN, D. H. 1969. Seed-eaters versus seed size, number, toxicity and dispersal. Evolution 23: 1–27.

_____. 1971. Escape of *Cassia grandis* L. beans from predators in time and space. Evolution 52: 964–979.

_____. 1981. The defences of legumes against herbivores. Pp. 951–978 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____, M. W. DEMMENT & J. B. ROBERTSON. 1985. How fast and why do germinating Guanacaste seeds *(Enterolobium cyclocarpum)* die inside cows and horses? Biotropica 17: 322–325.

JOHNSON, C. D. 1977. Ecology and behaviour of *Acanthoscelides mundulus* in seeds of *Nissolia schottii* (Coleoptera:Bruchidae; Leguminosae). Pan-Pacific Entomol. 53: 161–167.

_____. 1981a. Seed beetle host specificity and the systematics of the Leguminosae. Pp. 995–1027 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____. 1981b. Interaction between bruchid (Coleoptera) feeding guilds and behavioural patterns of pods of the Leguminosae. Entomol. Soc. Amer. 10: 249–253.

KELLY, K. M. & J. VAN STADEN. 1985. Effect of acid scarification on seed coat structure, germination and seedling vigour of *Aspalathus linearis*. J. Pl. Physiol. 121: 37–45.

KETRING, D. L. & P. W. MORGAN. 1969. Ethylene as a component of the emanation from germinating peanut seeds and its effect on dormant Virginia-type seeds. Pl. Physiol. 44: 326–330.

_____ & _____. 1970. Physiology of oil seeds. 1. Regulation of dormancy in Virginia-type peanut seeds. Pl. Physiol. 45: 269–273.

_____ & _____. 1971. Dormancy release in Virginia-type peanuts by plant growth regulators. Pl. Physiol. 47: 488–492.

KHUDAIRI, A. K. 1956. Breaking of dormancy of *Prosopis* seeds. Physiol. Pl. 9: 452–461.

KOPOOSHIAN, H. & D. ISELY. 1966. Seed character relationships in the Leguminosae. Proc. Iowa Acad. Sci. 73: 59–67.

LACKEY, J. A. 1981. Systematic significance of the epihilum in Phaseoleae (Fabaceae, Faboideae). Bot. Gaz. (Crawfordsville) 142: 160–164.

LEBEDEFF, G. A. 1943a. Heredity and environment in the production of hard seeds of common bean *(Phaseolus vulgaris)*. Puerto Rico Agric. Exp. Res. Bull. Number 4.

_____. 1943b. Inheritance of hard seed production in common beans *(Phaseolus vulgaris)*. Genetics 28: 80.

Lersten, N.R. 1981. Testa topography in Leguminosae, sub-family Papilionoideae. Proc. Iowa Acad. Sci. 88: 180–191.

_____. 1982. Tracheid bar and vestured pits in legume seeds (Leguminosae:Papilionoideae). Amer. J. Bot. 69: 98–107.

Lipp, A. E. G. & L. A. T. Ballard. 1959. The breaking of seed dormancy in some legumes by carbon dioxide. Austral. J. Agric. Res. 10: 495–499.

Manning, J. C. & J. Van Staden. 1985. The development and ultrastructure of the testa and tracheid bar in *Erythrina lysistemon* Hutch. (Leguminosae:Papilionoideae). Protoplasma 129: 157–167.

_____ & _____. 1987a. The systematic significance of testa anatomy in the Leguminosae—an illustrated survey. S. African J. Bot. 53: 210–230.

_____ & _____. 1987b. The role of the lens in seed and seedling vigour of *Sesbania punicea* (Cav.) Benth. (Leguminosae: Papilionoideae). Ann. Bot. (London) 59: 705–713.

Marbach, I. & A. M. Mayer. 1974. Permeability of seed coats to water as related to drying conditions and metabolism of phenolics. Pl. Physiol. 54: 817–820.

_____ & _____. 1975. Changes in catechol oxidase and the permeability to water in seed coats of *Pisum elatius* during seed development and maturation. Pl. Physiol. 56: 93–96.

Martin, J. N. 1922. The structure and development of the seed coat and causes of delayed germination in *Melilotus alba*. Proc. Iowa Acad. Sci. 29: 345–346.

Murray, D. R. 1979. Nutritive role of the seed coats during embryo development in *Pisum sativum* L. Pl. Physiol. 64: 763–769.

Nakamura, S. 1962. Germination of legume seeds. Proc. Int. Seed Testing Assoc. 27: 694–709.

Narasimhareddy, S. B. & P. M. Swamy 1979a. Abscisic acid-like inhibitors and cytokinins in the developing seeds of dormant and non-dormant varieties of peanut (*Arachis hypogaea* L.). J. Exp. Bot. 30: 37–42.

_____ & _____. 1979b. Abscisic acid-like inhibitors and cytokinins during after-ripening of dormant peanut seeds *(Arachis hypogaea)*. Physiol. Pl. 46: 191–193.

Naveh, Z. 1975. The evolutionary significance of fire in the Mediterranean region. Vegetatio 29: 199–208.

Noodén, L. D., K. A. Blakely & J. M. G. Crzybowski. 1985. Control of seed coat thickness and permeability in soybean. Pl. Physiol. 79: 543–545.

Nordhagen, R. 1959. Remarks on some new or little known myrmecochorous plants from North America and East Asia. Bull. Res. Council Israel, Sect. D, Bot. 7D: 184–201.

Nutile, G. E. & L. C. Nutile. 1947. Effect of relative humidity on hard seeds in garden beans. Proc. Assoc. Off. Seed Analysts N. Amer. 37: 106–114.

Patrick, J. W. 1983. Photosynthate unloading from seed coats of *Phaseolus vulgaris* L. General characteristics and facilitated transfer. Zeitschrift für Pflanzenphysiologie 111: 9–18.

Polhill, R. M. 1976. Genisteae (Adanson) Bentham and related tribes (Leguminosae). Bot. Syst. 1: 143–368.

Porter, R. H. 1949. Recent developments in seed technology. Bot. Rev. 15: 221–344.

Profumo, P., P. Gastaldo & R. Martinucci. 1979. On the inhibitory action of endosperm on germination of *Cercis siliquastrum* seeds. Experientia 35: 1452–1453.

Quinlivan, B. J. 1968. The softening of hard seeds of sand-plain lupin (*Lupinus varius* L.). Austral. J. Agric. Res. 19: 507–515.

_____. 1971. Seed coat impermeability in legumes. J. Austral. Inst. Agric. Sci. 37: 283–295.

Raleigh, G. J. 1930. Chemical conditions in maturation, dormancy and germination of seeds of *Gymnocladus dioica*. Bot. Gaz. (Crawfordsville) 89: 273–294.

Rees, B. 1911. Longevity of seeds and structure and nature of the seed coat and causes of delayed germination in *Melilotus alba*. Proc. Roy. Soc. Victoria 23: 293–414.

Reeve, R.M. 1946. Ontongeny of the sclereids in the integument of *Pisum sativum* L. Amer. J. Bot. 33: 806–816.

Reid, J. S. G. & J. D. Bewley. 1979. A dual role for the endosperm and its galactomannan reserves in the germinative physiology of fenugreek (*Trigonella foenum-graecum* L.), an endospermic leguminous seed. Planta 147: 145–150.

Riggio-Bevilacqua, L., G. Roti-Michelozzi & G. Serrato-Valenti. 1984. Water entry in *Cercis siliquastrum* (Leguminosae) seeds. Nordic J. Bot. 4: 675–679.

_____, _____ & _____. 1985. Barriers to water penetration in *Cercis siliquastrum* seeds. Seed Sci. & Technol. 13: 175–182.

ROBBERTSE, P. J. 1973. Die genus *Acacia* Miller in Suid Afrika—3 (met spesiale verwysing na die morfologie van die saad). Tydskrif vir Wetenskap en Kuns 13: 72–95.

SAHA, P. K. & N. TAKAHASHI. 1981. Seed dormancy and water uptake in *Crotalaria sericea* Retz. Ann. Bot. (London) 47: 423–425.

SALISBURY, E. J. 1942. The Reproductive Capacities of Plants: Studies in Quantitative Biology. G. Bell & Sons, London.

SERNANDER, R. 1906. Entwurf einer Monographie der europäischen Myrmekochoren. Kongl. Svenska Vetenskapsakad. Handl. 41: 1–410.

SHELDON, J. C. 1974. The behaviour of seeds in soil III. The influence of seed morphology and the behaviour of seedlings on the establishment of plants from surface-lying seeds. J. Ecol. 62: 47–66.

SLATTERY, H. D., B. J. ATWELL & J. KUO. 1982. Relationship between colour, phenolic content and impermeability in the seed coat of various *Trifolium subterraneum* L. genotypes. Ann. Bot. (London) 50: 373–378.

SPONSEL, V. M. 1983. The localization, metabolism and biological activity of gibberellins in maturing and germinating seeds of *Pisum sativum* cv. Progress No. 9. Planta 159: 454–468.

SPURNY, M. 1964. Changes in the permeability of the seed coat in connection with the development of suberin adcrustations of the macrosclereids from the seed coat of the pea (*Pisum sativum* L.) Flora 154: 547–567.

SREERAMULU, N. & I. M. RAO. 1969. Growth and endogenous gibberellin content of dormant groundnut embryonic axes as influenced by leaching and GA. Physiol. Pl. 22: 1134–1138.

STAHL, C. 1937. Report on the Danish State Seed Testing Station, 66th year. Tidsskr. Planteavl/Danish. J. Pl. & Soil Sci. 42: 553–619.

STERLING, C. 1954. Development of the seed coat of lima bean *(Phaseolus lunatus).* Bull. Torrey Bot. Club 81: 271–287.

SURI, R. K. & P. K. DESHPANDE. 1981. Development of seed in *Cassia absus* and C. *auriculata*. Phytomorphology 31: 40–47.

TANNER, W. 1980. On the possible role of ABA on phloem unloading. Ber. Deutsch. Bot. Ges. 93: 349–351.

THORNE, J. H. 1981. Morphology and ultrastructure of maternal seed tissue of soybean in relation to the import of photosynthate. Pl. Physiol. 67: 1016–1025.

———& R. M. RAINBIRD. 1983. An *in vivo* technique for the study of phloem unloading in seed coats of developing soybean seeds. Pl. Physiol. 72: 268–271.

TRIVEDI, B. S., G. D. BAGCHI & U. BAJPAL. 1979. Scanning electron microscopic studies on the sporoderm of some Mimosoideae (Leguminosae). Phytomorphology 29: 211–218.

TURCEK, F. J. 1963. Colour preference in fruit- and seed-eating birds. Proc. Thirteenth Int. Ornithological Congr. 1: 285–292.

TWAMLEY, B. E. 1967. Seed size and seedling vigor in birdsfoot trefoil. Canad. J. Pl. Sci. 47: 603–609.

VAN DER PIJL, L. 1982. Principles of Dispersal in Higher plants. Springer-Verlag, Berlin.

VAN STADEN, J., J. C. MANNING AND C. W. S. DICKENS. 1987. A phylogenetic analysis of the role of plant hormones in the development and germination of legume seeds. Pp. 387–442 *in* C. H. Stirton (editor), Advances in Legume Systematics.

WATSON, D. P. 1948. Structure of the testa and its relation to germination in the papilionoid tribes Trifoleae and Loteae. Ann. Bot. (London) 12: 385–409.

WERKER, E., A. DAFNI & M. NEGBI. 1973. Variability in *Prosopis falcata* in Israel: anatomical features of the seed. J. Linn. Soc., Bot. 66: 223–232.

———, I. MARBACH & A. M. MAYER. 1979. Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. Ann. Bot. (London) 43: 765–771.

WILLIAMS, W. A. 1967. Seedling growth of a hypogeal legume, *Vicia dasycarpa* in relation to seed weight. Crop Sci. 7: 163–165.

WHITE, J. 1908. The occurrence of an impermeable cuticle on the exterior of certain seeds. Proc. Roy. Soc. Victoria 21: 203–211.

WOLSWINKEL, P. & A. AMMERLAAN. 1983. Phloem unloading in developing seeds of *Vicia faba* L. The effect of several inhibitors on the release of sucrose and amino acids by the seed coat. Planta 158: 205–215.

——— & ———. 1984. Turgor-sensitive sucrose and amino acid transport into developing seeds of *Pisum sativum*. Effect of a high sucrose or mannitol concentration in experiments with empty ovules. Physiol. Pl. 61: 172–182.

——— & ———. 1985. Effect of potassium on sucrose and amino acid release from the seed coat of developing seeds of *Pisum sativum*. Ann. Bot. (London) 56: 35–43.

_____, _____& H. KUYVENHOVEN. 1983. Effect of KCN and p-chloromercuribenzenesulfonic acid on the release of sucrose and 2-amino {1-^{14}C} isobutyric acid by the seed coat of *Pisum sativum*. Physiol. Pl. 59: 375–386.

ZIMMERMAN, K. 1936. Zur physiologischen Anatomie der Leguminosentesta. Beiträge zum Problem der Hartschaligkeit und zur Bedentung des Strophiolums. Die Landw. Versuchsstat. 78: 1–56.

_____. 1942. Some experiments on the problem of hard seed coat in *L. angustifolius*. Züchter 14: 182–185.

MORPHOLOGY AND AERODYNAMICS OF WIND-DISPERSED LEGUMES

Carol K. Augspurger[1]

Abstract

A survey of the Leguminosae reveals that diaspores with morphological traits that appear to function in wind dispersal occur in approximately 22%, 42%, and 22% of the genera in the Caesalpinioideae, Mimosoideae, and Faboideae, respectively. Wind dispersal has undoubtedly had an independent origin in many different tribes. It occurs in all growth forms (herbs to trees) and from deserts to rainforests. Dry indehiscent fruits or segments of fruits are the diaspore in 94% of the genera; the rare winged seeds are restricted to the Mimosoideae and to two genera in the Caesalpinioideae. Although the diaspores of many species are single-seeded, some have multiple seeds. Seventeen morphological and seven aerodynamic categories of wind-dispersed diaspores occur in the family. Diaspores with different morphologies have different aerodynamic properties. These differences affect the rate of descent and stability when wind speeds change. Rate of descent can be predicted in part by the diaspore's (wing-loading)$^{1/2}$, i.e., (weight/projected area)$^{1/2}$. The relationship of rate of descent to wing-loading varies among morphological categories. Thus for a given wing-loading value, some diaspores move further than do others. Diaspores of representative species range in mass from 0.01 to 17.8 g, in projected surface area from < 1 to > 100 cm^2, in (wing-loading)$^{1/2}$ from 95 to 407 (millidynes·cm^{-2})$^{1/2}$, and in rate of descent from 91 to 368 cm/sec. Within a genus the area of the diaspore can vary greatly among species (e.g. 7 to 44 cm^2 in *Tachigali*). Provided the mass of the diaspore varies proportionately with area, then wing-loading and dispersal potential are unaffected. There is great interspecific variation in dispersal distance of wind-dispersed diaspores.

I. Introduction

Far less is known of the dispersal biology than of the breeding systems and pollination biology of the Leguminosae (Arroyo, 1981). The contributions of early naturalists, systematists, and plant ecologists have yielded a widely scattered literature on the dispersal biology of legumes, but no general synthesis has yet been made. Buchwold (1895) described the dispersal mechanisms of legumes in tropical Africa. Legume fruits have been classified according to evolutionary relationships (Dudik, 1981), and ecological (van der Pijl, 1956, 1972) and morphological (Gunn, 1984, in press) properties.

This review describes the taxonomic distribution of wind-dispersed diaspores in the Leguminosae. Morphological trends are summarized at the subfamily, tribe, and genus levels, and on the basis of growth form and general habitat. The aerodynamic responses of the various forms are then described. Finally the effects of mass, area, and morphology of different diaspores are examined in an attempt to evaluate their dispersal ability.

II. Methods

Those genera with at least one wind-dispersed species were identified. Determinations of pod morphology were made first on the basis of literature descriptions in Ridley (1930), van der Pijl (1956, 1972), Allen & Allen (1981), Polhill & Raven (1981), Gunn (1984, and in press), and a few floras. For the majority of genera, fruits were then observed first-hand from collections at the Field Museum Herbarium; U.S. National Herbarium, Smithsonian Institution; and particularly the U.S.

[1]Department of Plant Biology, 505 S. Goodwin, University of Illinois, Urbana, Illinois 61801 U.S.A.

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National Seed Herbarium, USDA/ARS, Systematic Botany, Mycology, and Nematology Laboratory. Additional information was drawn from field observations of wind-dispersed species in the Neotropics.

Because each of the 656 genera in the Leguminosae was not systematically reviewed, this summary is conservative. Some difficulty arose in inferring dispersal mode from the herbarium collections, and thus the categorization is viewed as preliminary. The strength of the conclusions for each genus is indicated in Appendix 1. Fruits categorized as simple samaras, i.e., flat, elongated pods, were particularly difficult because one could not always determine if and when the fruit became dehiscent, whether it was revolute (and hence explosively dispersed), and how thin and nonleathery the fruit must be to be a candidate for wind dispersal. Although any elongate flat fruit is probably dispersed at some time by strong winds, the issue is whether wind is the primary dispersal mode. Some pods included in this category may also be dispersed by water or ground mammals. Further field observations are needed to resolve this uncertainty.

III. Morphological Categories

The earliest legume fruit was probably folliclelike, dehiscent, and few-seeded; seeds probably were attached by the funiculus at the dorsal suture (Polhill et al., 1981). Specialization for wind dispersal proceeded in various ways with changes in: fruit dehiscence; shape, location, and number of wings; number and location of seeds; inflation or flattening of the pod; fruit segmentation; peeling off of exocarp and mesocarp before dispersal; and nonovarian (accessory) structures to aid dispersal. Evolutionary pathways leading to particular specializations are presently unknown.

The morphological categories presented in Table 1 and illustrated in Figure 1 are based on an expansion of the scheme of van der Pijl (1956), who recognized four groups of legumes with dry seeds and dry pericarps. The majority of the categories include indehiscence, which Dudik (1981) considered to be the derived condition. The two exceptions are dehiscent fruits dispersing individual *winged seeds* (IA), and *hemi-legumes* (IB) (notation throughout refers to Table 1; see also Fig. 1). In hemi-legumes the valves of the pod separate, and each valve becomes a diaspore carrying seeds. Comparative anatomical studies indicate that dehiscent and indehiscent pods differ in the orientation of their sclerenchymatous fibers and the presence of separation tissue between epidermal layers (Fahn & Zohary, 1955).

In genera with flat, winged seeds, fruit dehiscence occurs along one or two sutures. The shape of the seed and wing varies resulting in circular or elongated diaspores (IA1a, b). Some of the longest known seeds, greater than 10 cm including the wing, occur in this group. Some genera have only a winglike rim (under 2 mm wide) (IA2).

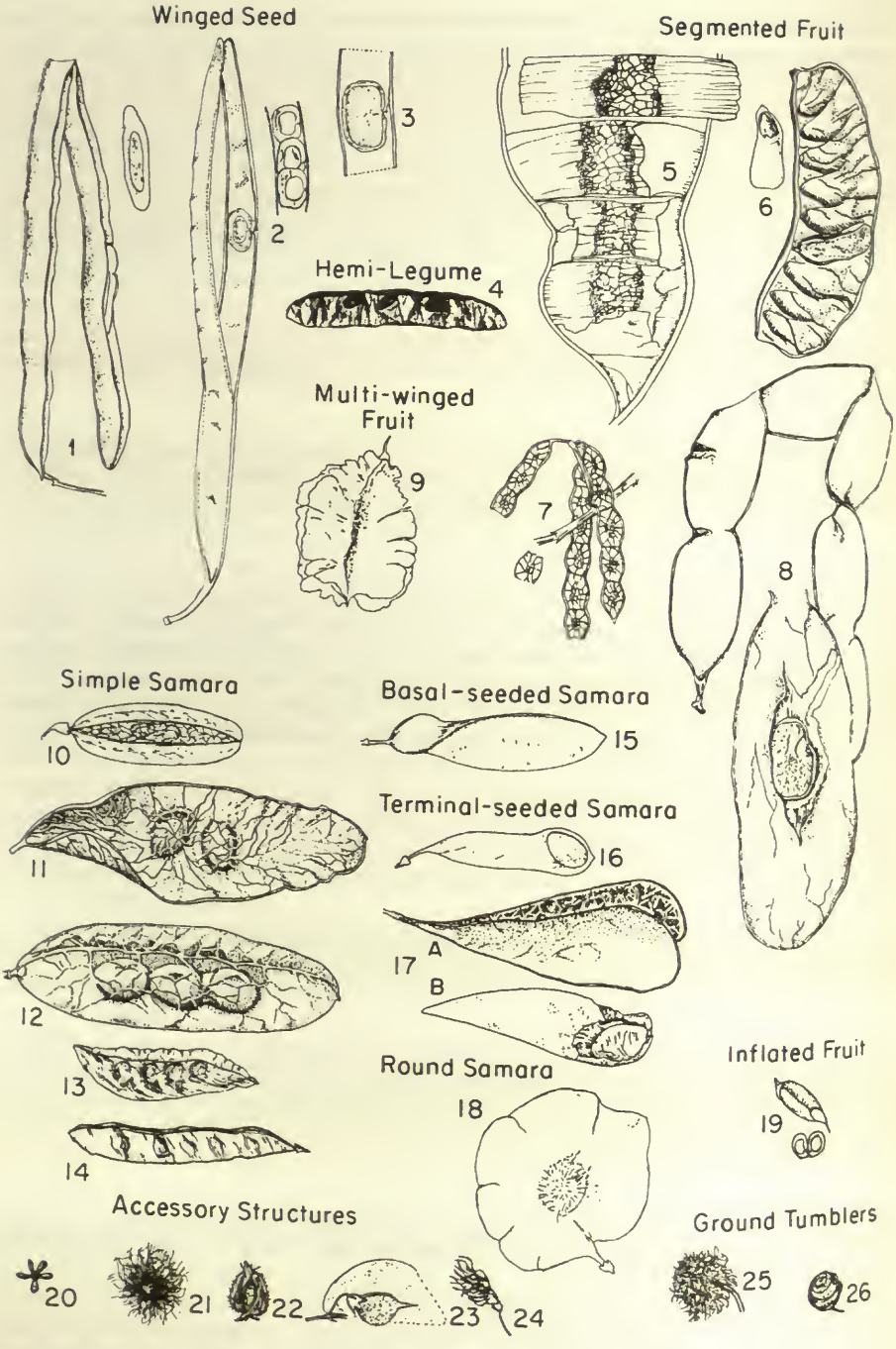
Indehiscent fruits occur in four main morphological groups: flat, multiwinged, cylindrical (inflated), and with accessories. The flat fruits are either entire (samaras) or segmented. Four forms of samaras differ in the shape and location of their seed(s). Their wings can be derived from stipe, valve, suture, or style base (Polhill et al., 1981). The most common form is the *simple samara*, an elongated, flat fruit with one central flat seed or several flat seeds throughout its length (IIA1a). The wing ranges from being almost nonexistent to having considerable development above the ventral suture (e.g., *Zenia*). Much rarer are equal wings from both sutures (e.g., *Gagnebina*). Only in one genus *(Ammodendron)* is the flat pod slightly twisted so as to resemble *Ailanthus* fruits. The *round samara* has a circular wing with a central flat seed(s) (IIA1b). The *basal-seeded samara* has a terminal wing (IIA1c), while the *terminal-seeded samara* has a basal wing (IIA1d). Both of these last types have spheroidal seeds. Some terminal-seeded samaras resemble winged seeds; but their wing is derived from the endocarp of the dehiscent fruit *(Schizolobium, Amburana)* (Gunn, 1981).

Some indehiscent flat fruits break into *segments* before dispersal (IIA2). Each segment with one central flat seed serves as the diaspore; the exocarp and mesocarp of the segment may (IIA2a) or may not (IIA2b) peel off before dispersal. The shape of the segment may be oblong or circular. The

TABLE 1. Morphological categories of wind-dispersed diaspores in the Leguminosae. The groups are arranged as a key. Also indicated are the taxonomic distributions within each subfamily, growth forms, shapes of seed, and numbers of seeds per diaspore. C = Caesalpinioideae; M = Mimosoideae; F = Faboideae.

	Total	C	M	F	Growth forms	Shapes of seeds	Numbers of seeds per diaspore
I. Dehiscent							
A. Winged seed	11	2	9		Nonherb	Flat	1
1. Wing > 2 mm	9	2	7				
a. Elongated seed	4		4				
b. Circular seed	5	2	3				
2. Wing < 2 mm	2		2				
B. Hemi-legume (each valve = diaspore)	4	1		3	Nonherb	Flat	1–few
II. Indehiscent							
A. Flat fruit							
1. Nonsegmented fruit (samara)							
a. Simple (elongated) samara	71	20	7	44	Nonherb	Flat	1–≥4
1 seed	33	7	1	25			
2 seeds	6	4		2			
3 seeds	6	2		4			
≥ 4 seeds	26	7	6	13			
b. Round samara	13	2	1	10	Nonherb, herb	Flat	1(2)
c. Basal-seeded samara (terminal wing)	11	2		9	Nonherb	Nonflat	1(2)
d. Terminal-seeded samara (basal wing)	12	6		6	Nonherb	Nonflat	1(2)
i. Fruit diaspore	10	5		5			
ii. Endocarp diaspore	2	1		1			
2. Segmented fruit	15	1	12	2	Nonherb	Flat	1
a. Abscission of endocarp segments	7	1	5	1			
b. Abscission of fruit segments	6		5	1			
c. Irregular break of fruit segments	2		2				
B. Multiwinged fruit	2			2	Nonherb	Nonflat	few–many
C. Cylindrical inflated fruit (balloons)	13			13	Herb	Nonflat	few
D. Accessory structures							
1. Calyx	6			6			
a. Expanded as wings	1			1	Nonherb	Nonflat	1
b. Pappus	1			1	Herb	Nonflat	?
c. Inflated balloon	4			4	Herb	Nonflat	few
2. Modified bract	1			1	Herb	Nonflat	1
3. Plumed pod	1			1	Herb	?	1/loment
4. Tumbleweeds	6			6	Herb	Nonflat	few–many
a. entire plant							
i. decay at stem base	2			2			
ii. abscission zone at stem base	1			1			
b. infructescence only	2			2			
c. fruit only	1			1			
Total wind-dispersed genera	159	34	27[1]	98[2]			
Total genera	656	152	64[3]	440			
% genera with wind-dispersal	24	22	42	22			

[1] *Albizia* occurs in 3 categories (27 + 2 = 29).
[2] *Astragalus* occurs in 4 categories and *Trifolium* in 3 categories (96 + 5 = 101).
[3] Number includes five unnamed genera (Gunn, 1984).


Winged Seed
Segmented Fruit
1
2
3
4
Hemi-Legume
5
6
Multi-winged Fruit
7
8
9
Simple Samara
10
11
12
13
14
Basal-seeded Samara
15
Terminal-seeded Samara
16
17
A
B
Round Samara
18
Inflated Fruit
19
Accessory Structures
20
21
22
23
24
Ground Tumblers
25
26

method of segmentation varies. In some genera abscission occurs along regular transverse zones; the sutures may or may not become part of the diaspore. Some genera lack an abscission zone and severing depends on an irregular break along constricted areas of the fruit (IIA2c). Particularly unusual is the derivation of the diaspore of *Melanoxylon* (Fig. 1). It resembles a basal-seeded samara, or a winged seed, but it is one segment of the endocarp of a fruit.

Multiwinged fruits with wings in more than one plane occur in two genera; the fruit of *Piscidia* is similar to that of *Cavanallesia* (Bombacaceae) or *Combretum* (Combretaceae). *Cylindrical* fruits arise as inflated papery balloons (IIC); most of these fruits are multiseeded.

Accessory structures (IID) are employed in dispersal in a limited number of genera. The persistent calyx is used in several ways. In *Apoplanesia* it expands into wings (IID1a) similar to those of *Cordia alliodora* (Boraginaceae). In *Trifolium* the calyx is pappuslike (IID1b). In several genera (e.g., *Anthyllis*), the calyx expands in a balloonlike structure (IID1c) surrounding the fruit. A paper-thin modified bract (IID2) in *Phylacium* surrounds the fruit. The segments of the pod in *Adesmia* produce long plumes (IID3). In some genera, either the plant (*Alhagi*) (IID4a), infructescence (*Trifolium*) (IID4b), or fruit (*Trifolium, Acacia*) (IID4c) tumbles across the ground.

The most common morphology is the simple samara; it occurs in 76 genera (Table 1). Several types of morphology are represented in a single genus. Fruits or segments of fruits are the diaspore in 94% of the genera; winged seeds are rare in the legumes.

IV. Seed Number per Diaspore

The number of seeds in a diaspore strongly affects its weight, aerodynamics, and rate of descent (see below). Seed number per diaspore also influences the level of seed predation and number of offspring establishing as seedlings adjacent to their siblings (e.g., Augspurger, 1986a). Dudik (1981) suggested an evolutionary trend toward reduction in seed number in the legumes. One-seeded diaspores are most common among wind-dispersed genera (Table 1), although some are multiseeded. One-seeded diaspores from multiseeded fruits arise if the diaspore is a winged seed or a fruit segment. One-seeded diaspores of entire fruits are common in samaras, especially in basal-seeded, terminal-seeded, and round types, although in each of these types a minority of diaspores are double-seeded (Augspurger, 1986a). Among simple elongated samaras seed number varies greatly (Table 1; Fig. 1); many genera have one-seeded diaspores, but an even greater number of genera have diaspores with ≥ 4 seeds (e.g., *Cercis*). Many of these pods are dehiscent after dispersal, and some of the seeds may be separated from the fruit after the initial dispersal event.

← Figure 1. Representatives of each major morphological category of wind-dispersed diaspores.

—1. (o) *Newtonia suaveolens* (winged elongated seed). —2. (o) *Indopiptadenia oudhensis* (winged circular seed). —3. (o) *Archidendropsis streptocarpa* (winged seed, < 2 mm rim). —4. *Robinia pseudoacacia* (hemilegume). —5. (o) *Entada polystachya*, (segmented fruit, endocarp segments). —6. (II) *Melanoxylon brauna* (a = fruit and b = endocarp segment with seed). —7. (o) *Piptadeniopsis lomentifera* (round segmented fruit segments). —8. (o) *Cedrelinga cateniformis* (elongated fruit segments, irregular break). —9. (Δ) *Piscidia grandifolia* (multiwinged fruit). —10. (Δ) *Hymenolobium petraeum* (simple 1-seeded samara). —11. (II) *Stemonocoleus micranthus* (simple 2-seeded samara). —12. (II) *Phyllocarpus pterocarpus* (simple 3-seeded samara). —13. (II) *Caesalpinia hymenocarpa* (simple 4-seeded samara). —14. (II) *Poeppigia procera* (simple 5-seeded samara). —15. (Δ) *Tipuana tipu* (basal-seeded samara). —16. (Δ) *Platypodium elegans* (terminal-seeded samara). —17. (II) *Schizolobium amazonicum*, (terminal-seeded samara, A = exocarp and mesocarp, B = endocarp with seed). —18. (Δ) *Pterocarpus erinaceus* (round samara). —19. (Δ) *Oxytropis campestris* (cylindrical inflated fruit). —20. *Apoplanesia cryptopetala* (calyx as wings). —21. *Trifolium pilulíferum* (calyx as pappus). —22. *Astragalus tortuosus* (calyx as inflated balloon). —23. (Δ) *Phylacium bracteosum* (modified bracts). —24. *Adesmia pedicellata* (plumed pod). —25. *Trifolium* sp. (infructescence ground tumbler). —26. *Medicago scutellata* (fruit ground tumbler). Drawings taken with permission from other sources are indicated by: o (Gunn, 1984); II (Gunn, in press); Δ (Polhill & Raven, 1981). All diaspores 1/3 actual size.

Individual plants with one-seeded simple samaras commonly have a low proportion of multiseeded samaras. Whether this variable number reflects variation in ovule number, pollination success, ovule abortion, and/or seed predation of fertilized ovules is unknown (see Bawa & Buckley, this volume). Multiseeded indehiscent fruits also arise in many of the herbaceous legumes, e.g., the inflated balloon-shaped fruits of *Lessertia* and *Sutherlandia*. Plants with an abscission zone near their stem base disperse their seeds as they tumble along the ground. These plants carry multiple seeds, although dispersal of single fruits and/or seeds may occur sequentially as they tumble.

V. Taxonomic Distribution

This initial survey estimates that wind dispersal occurs in at least one species in 158 (24%) genera of the Leguminosae; it occurs in an estimated 22%, 42%, and 22% of genera in the Caesalpinioideae, Mimosoideae, and Faboideae, respectively (Table 1). Wind dispersal occurs in four out of five tribes in the Caesalpinioideae, four out of five tribes in the Mimosoideae, and 19 out of 31 tribes in the Faboideae (Appendix I). Large numbers of wind-dispersed genera are represented in the tribes Caesalpinieae, Cassieae, Detarieae, Mimoseae, Dalbergieae, Galegeae, Sophoreae, and Tephrosieae.

Morphological convergence occurs among subfamilies (Table 1) and in many tribes within a subfamily (Appendix I). Some morphological categories are restricted to one or two subfamilies (Table 1). Winged seeds occur only in the Mimosoideae and in two genera of the Caesalpinioideae (*Campsiandra* and *Diptychandra*). Basal-seeded and terminal-seeded samaras are not found in the Mimosoideae. Inflated fruits and accessory structures are restricted to the Faboideae, the subfamily in which most temperate herbs occur. The exception for accessory structures being associated with temperate herbs is the tropical tree, *Apoplanesia*, whose fruits are half-included within expanded calyx wings. In total, the Faboideae are represented by the most morphological categories and the Mimosoideae by the least. Dudik (1981) suggested that this type of evidence supports the hypothesis that the Faboideae are the most advanced group.

The taxonomic units at the tribal level are usually determined by floral structure; it is less common to distinguish genera using fruit structure as a criterion. The obvious features, such as wings or indehiscence, may arise as readily within species or genera as between them (Polhill et al., 1981). Morphological specializations of fruits are more often restricted to the level of genus or species and not to tribe. Within a tribe it is most common to have a variety of morphological categories; exceptions to this generalization are the Thermopsideae, which are represented by only one morphology, and the Galegeae, which are heavily dominated by one morphology (Appendix I).

Usually only one type of wind-dispersed diaspore occurs in a genus. A few species-rich genera, such as *Albizia*, *Astragalus*, and *Trifolium*, have more than one type of wind-dispersed diaspore. Ridley (1930) reported basal-seeded and terminal-seeded samaras in one species, *Derris robusta*. This difference in seed location may reflect differences in pollination or abortion patterns in a multiovulate ovary (see Bawa & Buckley, this volume).

VI. Ecological Correlates with Wind Dispersal

The distribution of morphological categories along taxonomic lines varies according to growth form, plant height, or geographic distribution. The majority of Caesalpinioideae and Mimosoideae are woody and tropical; the Faboideae are divided roughly into woody tropical and herbaceous temperate groups (Table 1). The wind-dispersed genera in all three subfamilies in tropical habitats are characterized largely by having indehiscent samaras, hemi-legumes, segmented fruits, or dehiscent fruits with winged seeds (Table 1). The morphological categories found among temperate herbaceous genera in the Faboideae show almost no overlap with those found among tropical trees,

shrubs, and vines. These herbaceous genera have evolved unique cylindrical inflated fruits or the use of accessory structures (Table 1). This dichotomy in morphological forms between herbaceous and nonherbaceous plants is related to aerodynamic limitations imposed by plant height (see below).

In tropical habitats the wind-dispersed woody legumes coexist with many non-wind-dispersed legumes, sometimes within the same genus. In general, an increase in the proportion of wind-dispersed species in tropical forests is correlated with a decrease in annual rainfall (Howe & Smallwood, 1982). Communities with lower rainfall have a more open structure, thus enhancing wind penetration. Indeed in closed, wet, tropical forests, wind-dispersed species are restricted to subcanopy, canopy, and emergent trees and lianas that have access to strong winds (pers. obs.).

Whitehead (1983) has recently reviewed environmental factors contributing to the frequency of wind pollination in a community. Wind dispersal of the Leguminosae is not linked to pollination syndromes (van der Pijl, 1972). Wind pollination and wind dispersal almost never co-occur in this family (but see *Ateleia herbert-smithii,* Janzen, this volume; *Hardwickia,* van der Pijl, 1972).

Dispersal structures may be correlated with aspects of life history. In a survey of the dispersal structures in the Asteraceae, Venable & Levin (1983) found that a lower percentage of annual species than perennial species have dispersal structures, and plumed types are more frequent in perennials, whereas adhesive types are more frequent in annuals. In the Asteraceae, differences in the nature of dispersal structures among perennial herbs, shrubs, and trees are minor. A comparable analysis of life history has not been done for the Leguminosae.

VII. Aerodynamic Consequences of Morphological Design

The morphology of the wind-dispersed diaspore dictates its aerodynamic motion. The many morphological designs found in the Leguminosae (Fig. 1; Table 1) represent a restricted number of aerodynamic categories (Table 2). No aerodynamic motion is unique to this family; the categories parallel those found earlier among 16 families in a tropical lowland forest (Augspurger, 1986b) except for the diaspores, such as inflated balloons, tumbleweeds, and tumbling fruits, that roll along the ground in open habitats.

Observing a diaspore falling in still air is a first approach to understanding its aerodynamic motion and predicting its dispersal ability. The biomechanics of a diaspore's motion in a fluid medium such as air are described by Norberg (1973), McCutchen (1977), and Vogel (1981). A falling diaspore always is affected by two forces, net body force and drag. Net body force is weight minus buoyancy. Drag is the rate of removal of momentum from air by the diaspore; its value is increased by increasing the projected area of the diaspore, thus lowering its velocity of fall. All falling diaspores experience drag (Table 2). In some cases lift also occurs due to the motion of the diaspore around some axis. Lift refers to the upward force generated when a diaspore rotates as it falls; its value is increased by an increase in the diaspore's rate of rotation, thus decreasing its velocity of fall. Only some diaspores experience lift, i.e., the autogyro, rolling autogyro, and helicopter (Table 2). The true glider, capable of forward momentum in still air, is lacking in the Leguminosae, although undulators (Table 2) glide for a portion of their flight.

It is difficult to translate the aerodynamic motion in still air to that experienced under field conditions. Likewise, no attempt has been made to predict what categories of motion are expected in habitats varying in wind speed and turbulence. The glider and perhaps the undulator are the least stable in turbulent air (McCutchen, 1977). Autorotation or autogyration (see Table 2) improves stability in turbulent air and diaspores combining both motions, i.e., rolling autogyros, are expected to be the most stable (Green, 1980a).

All morphologies of wind-dispersed diaspores affect their motion in ways that slow their rates of descent and thus increase their chances of exposure to horizontal winds. Studies by Green (1980b) and Augspurger (1986b) show that a diaspore's rate of descent is generally proportional to its $(\text{wing-loading})^{1/2}$; where wing-loading is defined as weight/projected area. However, the relation-

TABLE 2. A description of the aerodynamic forces and behaviors of the basic aerodynamic groups represented in the wind-dispersed Leguminosae. Also indicated are the morphological groups from Table 1 and Appendix I that correspond to the various aerodynamic groups.

Aerodynamic groups	Morphological groups	Aerodynamic behavior in still air
Lift and drag forces		
Helicopter	Calyx expanded as wings	Spins tightly around a vertical line; similar to autogyro with added wings
Autogyro	Basal-seeded samara Terminal-seeded samara	Rotates tightly around the seed end of the diaspore
Rolling autogyro	Simple samara Hemi-legume Elongated diaspore from segmented fruit Elongated winged seed	Rotates on two axes simultaneously: 1) around diaspore's longitudinal axis; 2) around one end of the diaspore in a semitight spiral
Drag force only		
Undulator	Round samara Round diaspores from segmented fruit Round winged seed	Glides and undulates but not with cumulative forward motion (not continuous glider or fugoid oscillator)
Tumbler	Multiwinged fruit	Tumbles randomly; also rotates in a large spiral around a vertical line
Floater	Calyx expanded as pappus Plumed pod (loment) Round inflated fruit Inflated calyx Modified bract	Floats downward in a vertical line; last three also may roll across ground in open habitats
Nonaerial	Tumbleweed	Rolls across ground in open habitats

ship of rate of descent to wing-loading varies among aerodynamic categories. For example, at a given wing-loading value, either the floating plumed pod of *Adesmia* or the tumbler of *Piscidia* is expected to fall faster than other types of diaspores because its design produces drag but no lift. In contrast, designs such as autogyros (basal- or terminal-seeded samaras), rolling autogyros (simple samaras), and helicopters (Table 2) produce both drag and lift forces and thus fall at slower rates. Over most of the range of values for wing-loading, rolling autogyros fall faster than autogyros (Augspurger, 1986b).

The growth form and height of the plant restrict the type of aerodynamic motion possible. For example, a period of free fall occurs before a diaspore achieves its autogyration or autorotation and begins to produce the lift force. In contrast, drag force begins immediately after the fall begins. Short herbaceous plants have diaspore morphologies that do not use lift, which is found in tall shrubs and trees (Tables 1, 2).

The distance of free fall, before autogyration, is correlated with blade length (Guries & Nordheim, 1984); long blades require a longer initial fall than do short blades to swing through to a trailing position. In their study of 55 species of *Acer*, the height of mature trees was positively correlated with the blade length of samaras. In the Leguminosae, plant height is expected to be related similarly to morphological dimensions. Blade length in the terminal-seeded samara of *Platypodium elegans* is 12 cm on a 30 m tree in Panama but only 6 cm on a 4 m tree in Brazil (Augspurger, unpublished data).

Autorotation produces the most lift when the diaspore is very flat. The seed of rolling autogyros is invariably flattened, as is the seed of undulators (Table 1). In contrast, autogyration is less affected by a swollen end nearest its rotation point; autogyros and all remaining designs have more spheroidal seeds (Table 1).

Autogyros include samaras with either basal or terminal seeds. The consequence of this contrast in seed location for aerodynamic motion is unknown. Having a heavy seed away from rather than near to the attachment point on the plant may influence the force required to sever the diaspore from the plant. Also it may influence how quickly the heavy end is oriented downwards as it falls; this orientation is a precondition for initiating autogyration.

The wind force required to sever the diaspore from the parent will have a strong effect on dispersal. Quantification of this force is unavailable for any wind-dispersed diaspore. Undoubtedly the strength of attachment varies with changes in humidity and through time. Species with clear abscission zones, e.g., segmented fruits, or with winged seeds attached by a thin funiculus, may require less force than species requiring irregular breakage of wide fruit tissue (e.g., *Cedrelinga*, *Platypodium*).

VIII. Effects of Mass, Area, and Morphology on Dispersal Ability

Predictions of a diaspore's dispersal ability can be made if values are known for the height of release, mean wind speed during dispersal, and rate of descent in still air (Cremer, 1971). Mass and projected area of the diaspore, together with morphology, are the factors most strongly determining the diaspore's rate of descent.

Measurements of mass must be made on freshly collected diaspores. Herbarium collections commonly have immature specimens or mature specimens whose mass has been altered by dehydration or respiration. As a result, only a limited number of mass and wing-loading values are available for the Leguminosae. Representative species range in mass from 0.01 to 17.8 g, in projected surface area from less than 1 to over 100 cm^2, in $(\text{wing-loading})^{1/2}$ from 95 to 407 $(\text{millidynes}\cdot cm^{-2})^{1/2}$, and in rate of descent from 91 to 368 cm/sec; see Augspurger (1986b) for more detailed values for a sample of tropical woody legume species. The maximum values recorded are for *Centrolobium* whose autogyro appears to be the largest wind-dispersed diaspore in any family.

In all wind-dispersed diaspores, development that enlarges the area exposed to the wind begins after fertilization and occurs in the ovary, seed coat, or such accessory structures as the calyx. An exception could be argued for the segmentation of fruits in which the smaller one-seeded segment becomes the diaspore and the area of the diaspore is less than the expanded fruit. Here it appears that one-seededness is more important than area.

Diverse mechanisms reduce the diaspore's mass before dispersal. First, major dehydration of tissues occurs. In a set of eight tropical legumes, the moisture content of freshly dispersed diaspores averaged 13.4% with a range of 8–32% (Augspurger, 1988). These values are much lower than the water content of most plant tissues (80–95% according to Street & Opik, 1975). Second, mass is reduced by the shedding of parts before dispersal, e.g., peeling off of the exocarp and/or mesocarp. Third, only portions of the initial fruit become the diaspore; this occurs in segmented fruits in which each one-seeded segment is the diaspore, in hemi-legumes in which each valve moves independently, and in winged seeds that leave all fruit tissue behind.

Fruit diaspores are usually much heavier than seed diaspores (Augspurger, 1988). The mass found in nonseed components of diaspores, i.e., tissues whose function is seed protection or dispersal can be quite high, especially in fruit diaspores of the Leguminosae. In a set of eight tropical fruit diaspores the percent of the diaspore's mass concentrated in the seed alone averaged only 33%. This contrasts with 61% for a group of 34 species that included nonlegumes and many seed diaspores (Augspurger, 1988).

An extreme reduction in mass occurs when seed number is lower, although in simple samaras this is often accompanied by a reduction in fruit area, thus offsetting the lowering of wing-loading achieved by weight reduction. The genera with simple samaras show a wide range in seed number

(Fig. 1; Appendix I). In addition to variation among genera, seed number can also vary among fruits of an individual tree. The legume *Lonchocarpus pentaphyllus* matures fruits containing 0–4 seeds. The area of the fruit does not vary proportionately and wing-loading and rate of descent increase with added seed number. Seed mass decreases as seed number increases. Fruits with more seeds are dispersed shorter distances. However, a greater proportion of two- and three-seeded fruits have at least one intact mature seed escaping seed predation than do one-seeded fruits. This may explain why variation in seed number remains even at the loss of some dispersal distance (Augspurger & Hogan, 1983). A second example occurs in the legume *Platypodium elegans* which produces one- and two-seeded fruits (Augspurger, 1986a). The double-seeded fruits are dispersed shorter distances. Seedlings closer to the parent suffer higher levels of pathogen mortality (Augspurger, 1983). The proximal seed of two-seeded fruits weights less, has lower germination, and its radicle has difficulty in emerging from the legume. Twin seedlings from one fruit grow slower, and both seedlings rarely survive one year. Unless two-seeded legumes of *Platypodium* incur less seed predation, the double-seeded nature of this species appears to be disadvantageous.

The independent influence of mass and area on dispersal distance was demonstrated recently in a field experiment using artificial fruits that mimicked the design of the simple one-seeded samara of the legume, *Tachigali versicolor* (Augspurger & Franson, 1987). The models were made using stiff paper for the fruit and parafilm for the seed. They were designed to vary independently in seed mass alone, fruit area alone, or simultaneously in mass and area so as to maintain constant wing-loading. All the models were released simultaneously from a 40-m platform above the forest canopy on Barro Colorado Island, Panama. The model fruits with the lowest wing-loading (achieved by decreasing seed mass or increasing fruit area) were dispersed the greatest mean distance, were scattered most evenly, and over the broadest area. Model fruits with widely varying area but with no difference in wing-loading had equal dispersal ability.

Many legume genera have broad geographical distributions. They display considerable variability in the surface area of their fruits. For example, the surface area of fruits of *Tachigali (Sclerolobium)* range from 7 to 44 cm^2 and those of *Platypodium* range from 4 to 31 cm^2. The modeling experiment demonstrated that an evolutionary change that modifies surface area accompanied by a change in mass that maintains a constant wing-loading should not affect the diaspore's dispersal distance. Data are needed to test how mass and wing-loading values change over the geographical range of a genus.

Significant variation in wing-loading occurs among 20 trees of the tropical legume *Platypodium elegans* on Barro Colorado Island: 240–319 $(\text{millidynes}\cdot\text{cm}^{-2})^{1/2}$ (Augspurger, unpublished). Field experiments, simultaneously releasing fruits from all parents from a common height, revealed that seeds from parents with lower wing-loading values were dispersed to greater distances. However, when measurements were made of the distribution of naturally dispersed fruits for 7 of the 20 trees, it was found that tree height rather than wing-loading and rate of descent in still air was particularly important for explaining the extreme tail of the distributions, i.e., whether maximum distance was 38 or 105 m from the parent tree (Augspurger, unpublished).

The experiment using artificial fruits also demonstrated the importance of morphology, independent of wing-loading, on dispersal ability. Terminal-seeded samaras of *Platypodium elegans* can have the same surface area as the simple samaras of *Tachigali versicolor;* mean wing-loading of *Platypodium* is 262 $(\text{millidynes}\cdot\text{cm}^{-2})^{1/2}$ while that of *Tachigali* is 178 $(\text{millidynes}\cdot\text{cm}^{-2})^{1/2}$ (Augspurger, 1986b). Artificial models of *Platypodium* were made that reduced seed mass so their mean wing-loading value and area equaled that of *Tachigali.* At the artificially lower wing-loading value *Platypodium* was unable to achieve its autogyration and it plummeted to the ground. As seed mass was augmented experimentally to normal levels for *Platypodium,* its autogyration was restored (Augspurger & Franson, 1987). The dilemma is that while added mass is essential for aerodynamic purposes, it results in increased wing-loading and causes a major loss in dispersal distance.

IX. Concluding Remarks

This preliminary survey, based largely on inference from herbarium collections, indicates the need for further field observations and measurements to verify which genera have wind-dispersed diaspores. The large number and variety of wind-dispersed diaspores found in leguminous plants suggests that during the course of evolution a variety of solutions to similar ecological problems have evolved within the family. Wind-dispersal undoubtedly has had many independent origins within the family. Similar types of surveys are needed for other families and other dispersal modes. Leguminous species using wind dispersal exist in most habitats; they coexist with leguminous species using animal-dispersed modes and abiotic modes, such as explosive and water dispersal. The challenge is to understand what selective pressures favor wind dispersal and what determines the different morphological designs and their aerodynamics.

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References

Allen, O. N. & E. K. Allen. 1981. The Leguminosae. Univ. of Wisconsin Press, Madison, Wisconsin.

Arroyo, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–770 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Augspurger, C. K. 1983. Seed dispersal of the tropical tree, *Platypodium elegans,* and the escape of its seedlings from fungal pathogens. J. Ecol. 71: 759–771.

———. 1986a. Double- and single-seeded indehiscent legumes of *Platypodium elegans:* consequences for wind dispersal and seedling growth and survival. Biotropica 18: 45–50.

———. 1986b. Morphology and dispersal potential of wind-dispersed diaspores of neotropical trees. Amer. J. Bot. 73: 353–363.

———. 1988. Mass allocation, moisture content, and dispersal capacity of wind-dispersed tropical diaspores. New Phytol. 108: 357–368.

——— & S. E. Franson. 1987. Wind dispersal of artificial fruits varying in mass, area, and morphology. Ecology 68: 27–42.

——— & K. P. Hogan. 1983. Wind dispersal of fruits with variable seed number in a tropical tree (*Lonchocarpus pentaphyllus:* Leguminosae). Amer. J. Bot. 70: 1031–1037.

Buchwold, J. 1895. Die Verbreitungsmittel der Leguminosen des tropischen Afrika. Bot. Jahrb. Syst. 19: 494–561.

Cremer, K. W. 1971. Speeds of falling and dispersal of seed of *Pinus radiata* and *Pinus contorta.* Austral. Forest Res. 5: 29–32.

Dudik, N. M. 1981. Morphology of the pods of Leguminales (Fabales). Pp. 897–901 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Fahn, A. & M. Zohary. 1955. On the pericarpal structure of the legumen, its evolution and relation to dehiscence. Phytomorphology 5: 99–111.

Green, D. S. 1980a. The Aerodynamics and Dispersal of Ash, Tuliptree, and Maple Samaras. Ph.D. dissertation. Princeton Univ., Princeton, New Jersey.

———. 1980b. The terminal velocity and dispersal of spinning samaras. Amer. J. Bot. 67: 1218–1224.

GUNN, C. R. 1981. Seeds of Leguminosae. Pp. 913–925 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

_____. 1983. A nomenclator of Legume (Fabaceae) genera. Techn. Bull. U.S.D.A. 1680: 1–224.

_____. 1984. Fruits and seeds of genera in the subfamily Mimosoideae (Fabaceae). Techn. Bull. U.S.D.A. 1681: 1–194.

_____. Fruits and seeds of genera in the subfamily Caesalpinioideae (Fabaceae). Techn. Bull. U.S.D.A. (in press).

GURIES, R. P. & E. V. NORDHEIM. 1984. Flight characteristics and dispersal potential of maple samaras. Forest Sci. 30: 434–440.

HOWE, H. F. & J. SMALLWOOD. 1982. Ecology of seed dispersal. Annual Rev. Ecol. Syst. 13: 201–228.

MCCUTCHEN, C. W. 1977. The spinning rotation of ash and tulip tree samaras. Science 197: 691–692.

NORBERG, R. A. 1973. Autorotation, self-stability, and structure of single-winged fruits and seeds (samaras) with comparative remarks on animal flight. Biol. Rev. Cambridge Philos. Soc. 48: 561–596.

POLHILL, R. M. & P. H. RAVEN (editors). 1981. Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____, _____& C. H. STIRTON. 1981. Evolution and systematics of the Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

RIDLEY, H. N. 1930. The Dispersal of Plants Throughout the World. L. Reeve & Co., Ashford, England.

STREET, H. E. & H. OPIK. 1975. The Physiology of Flowering Plants. American Elsevier Publishing Co., New York.

VAN DER PIJL, L. 1956. Classification of the leguminous fruits according to their ecological and morphological properties. Proceedings K. Nederlandse akademie van Wetenschappen (C) 59: 301–313.

_____. 1972. Principles of Dispersal in Higher Plants. Springer-Verlag, New York.

VENABLE, D. L. & D. A. LEVIN. 1983. Morphological dispersal structures in relation to growth habit in the Compositae. Pl. Syst. Evol. 143: 1–16.

VOGEL, S. 1981. Life in Moving Fluids. The Physical Biology of Flow. Willard Grant Press, Boston.

WHITEHEAD, D. R. 1983. Wind pollination: some ecological and evolutionary perspectives. Pp. 97–109 *in* L. Real (editor), Pollination Biology. Academic Press, New York.

APPENDIX 1. List of genera with at least one wind-dispersed species. Genera are organized by tribe within each subfamily; taxonomy follows Gunn (1983). Degree of confidence in conclusion about wind dispersal is indicated by (+) = certain, (+?) = probable, and (?) = possible. Also indicated are the morphological categories, numbers of seeds per diaspore, and growth forms of the wind-dispersed taxa. Numbers in parentheses refer to the minority seed numbers.

		Morphology	Numbers of seeds per diaspore	Growth forms H = herb N = nonherb
CAESALPINIOIDEAE				
CAESALPINIEAE				
Burkea	+	simple samara	1	N
Caesalpinia	?	simple samara	(1)–>4	N
Campsiandra	?	winged seed (round)	1	N
Diptychandra	+	winged seed (round)	1	N
Haematoxylum	+	simple samara	1–3	N
Melanoxylon	+	endocarp segments	1	N
Peltophorum	?	simple samara	1–4	N
Poeppigia	+?	simple samara	2–6	N
Pterogyne	+	basal-seeded samara	1	N
Pterolobium	+	basal-seeded samara	1	N
Recordoxylon	?	simple samara	2–7	N
Schizolobium	+	terminal-seeded samara (endocarp only)	1	N

APPENDIX 1. Continued.

		Morphology	Numbers of seeds per diaspore	Growth forms H = herb N = nonherb
Sclerolobium	+	simple samara (endocarp only)	1(2)	N
Tachigali	+	simple samara (endocarp only)	1(2)	N
Tetrapterocarpon	+	round samara (4 wings; 2 greatly reduced)	1	N
CASSIEAE				
Apuleia	+?	round samara?	1–2	N
Distemonanthus	+?	simple samara	2–5	N
Kalappia	?	simple samara	1–3	N
Koompassia	+	simple samara	1	N
Martiodendron	+	simple samara	1	N
Storckiella	+?	simple samara	1–8	N
Zenia	+?	simple samara	4	N
CERCIDEAE				
Adenolobus	+?	simple samara?	3	N
Bauhinia	+	simple samara	(1)2	N
Cercis	+	simple samara	≥4	N
DETARIEAE				
Apaloxylon	+	terminal-seeded samara	1	N
Bathiaea	+	terminal-seeded samara	1	N
Daniellia	+	hemi-legume (terminal-seeded)	1	N
Gossweilerodendron	+	terminal-seeded samara	1	N
Hardwickia	+	terminal-seeded samara	1	N
Hylodendron	+	simple samara?	1(2)	N
Oxystigma	+	terminal-seeded samara	1	N
Phyllocarpus	+	simple samara	1–3	N
Stemonocoleus	+	simple samara	1(2)	N
MIMOSOIDEAE				
ACACIEAE				
Acacia	+	simple samara; "tumbleweed" (fruit only)	>4	N
INGEAE				
Albizia	+	endocarp segment; fruit segment; simple samara	1 or >4	N
Archidendropsis	?	winged seed (<2 mm rim)	1	N
Cedrelinga	+	fruit segment (irregular break)	1	N
Pithecellobium	+?	fruit segment	1	N
Wallaceodendron	+	endocarp segment	1	N
MIMOSEAE				
Anadenanthera	?	winged seed (<2 mm rim)	1	N
Aubrevillea	+	fruit segment (irregular break)	1	N
Cylicodiscus	+	elongated winged seed	1	N
Dinizia	?	simple samara	>4	N
Entada	+	endocarp segment	1	N
Fillaeopsis	+	elongated winged seed	1	N

Appendix 1. Continued.

		Morphology	Numbers of seeds per diaspore	Growth forms H = herb N = nonherb
Gagnebina	+	simple samara (double-winged)	>4	N
Indopiptadenia	+	circular winged seed	1	N
Leuceana	?	simple samara	>4	N
Lysiloma	?	simple samara	>4	N
Mimosa	+?	fruit segment	1	N
Monoschisma	+	circular winged seed	1	N
Newtonia	+	elongated winged seed	1	N
Parapiptadenia	+	circular winged seed	1	N
Piptadeniastrum	+	elongated winged seed	1	N
Piptadeniopsis	?	fruit segment	1	N
Plathymenia	+	endocarp segment	1	N
Prosopidastrum	?	fruit segment	1	N
Pseudoentada	+	endocarp segment	1	N
Xerocladia	+	round samara	1	N
MIMOZYGANTHEAE				
Mimozyganthus	+	simple samara	1	N
FABOIDEAE				
ADESMIEAE				
Adesmia	+	plumed pod (loment)	1	H
AESCHYNOMENEAE				
Belairia	+?	simple samara	2–3	N
Chaetocalyx	?	fruit segments	1	N
Discolobium	?	round samara	1	H
Nissolia	+	basal-seeded samara	1–5	N
AMORPHEAE				
Apoplanesia	+	calyx wings	1	N
Dalea	?	inflated calyx	1	H
Eysenhardtia	?	simple samara?	1	N
CARMICHAELIEAE				
Carmichaelia	?	simple samara	>4	N
Streblorrhiza	?	simple samara	2–3	N
CROTALARIEAE				
Wiborgia	?	round samara	1–3	N
DALBERGIEAE				
Cascaronia	+	simple samara	1	N
Centrolobium	+	basal-seeded samara	1(3)	N
Dalbergia	+	simple samara	1(4)	N
Fissicalyx	+	round samara?	1	N
Grazielodendron	+	simple samara	1	N
Hymenolobium	+	simple samara	1(2)	N
Machaerium	+	basal-seeded samara	1	N
Paramachaerium	+	basal-seeded samara	1(3)	N
Platymiscium	+	simple samara	1	N
Platypodium	+	terminal-seeded samara	1(2)	N
Pterocarpus	+	round samara	1(3)	N
Riedeliella	+	round samara	3–4	N
Tipuana	+	basal-seeded samara	1(3)	N
Vatairea	+	basal-seeded samara	1	N
Vataireopsis	+	basal-seeded samara	1	N

Appendix 1. Continued.

		Morphology	Numbers of seeds per diaspore	Growth forms H = herb N = nonherb
DESMODIEAE				
Phylacium	+?	modified bract	1	H (vine)
DIPTERYGEAE				
Pterodon	+	simple samara (endocarp only)	1	N
GALEGEAE				
Alhagi	+	tumbleweed (decay at stem base)	>4	H
Astragalus	+	hemi-legume; inflated fruit; inflated calyx; tumbleweed (decay at stem base)	>4	H
Clianthus	+?	inflated fruit	>4	H
Colutea	+	inflated fruit	>4	(N) low shrub
Eremosparton	+?	inflated fruit	1–2	(N)
Halimodendron	+	inflated fruit	>4	(N)
Lessertia	+	inflated fruit	1–>4	H
Oreophysa	+?	inflated fruit	4	?
Oxytropis	+	inflated fruit	>4	H
Smirnowia	+	inflated fruit	>4	(N)
Sphaerophysa	+	inflated fruit	>4	H
Sutherlandia	+	inflated fruit	>4	(N)
Swainsonia	+	inflated fruit	>4	H
HEDYSAREAE				
Onobrychis	+?	round samara	1–3	H
LIPARIEAE				
Hypocalyptus	?	simple samara; inflated fruit	>4	N
LOTEAE				
Anthyllis	+	inflated calyx	2–>4	H
Hymenocarpus	+?	round samara	1–2	H
PHASEOLEAE				
Butea	+	terminal-seeded samara	1	N
Erythrina	+	hemi-legume	>4	N
Mastersia	?	simple samara	>4	N
Spatholobus	+	terminal-seeded samara	1	N
PSORALEEAE				
Orbexilum	+	tumbleweed (ascission zone at base of plant)	>4	H
Pediomelum	+	"tumbleweed" (infructescence only)	1	H
ROBINIEAE				
Diphysa	+?	inflated fruit	>4	(N)
Robinia	+	hemi-legume	>4	N
Sesbania	?	multiwinged fruit	>4	N
SOPHOREAE				
Acosmium	+	simple samara	1(4)	N
Amburana	+	terminal-seeded samara (endocarp only)	1(2)	N

APPENDIX 1. Continued.

		Morphology	Numbers of seeds per diaspore	Growth forms H = herb N = nonherb
Ammodendron	+?	simple samara (twisted)	1(2)	N
Amphimas	+	simple samara	1(2)	N
Ateleia	+	round samara?	1	N
Bolusanthus	+	simple samara	1–5	N
Bowdichia	+	simple samara	1(4)	N
Calpurnia	+	simple samara	1–7	N
Cladrastis	+?	simple samara	4–6	N
Dicraeopetalum	+	simple samara	1(3)	N
Diplotropis	+	simple samara	1	N
Lovanafia	+	simple samara	1(2)	N
Luetzelburgia	+	basal-seeded samara	1	N
Maackia	+	simple samara	1–5	N
Myrocarpus	+	simple samara	1(2)	N
Myrospermum	+	terminal-seeded samara	1	N
Myroxylon	+	terminal-seeded samara	1	N
Pericopsis	?	simple samara	1–7	N
Petaladenium	?	simple samara	4–5	N
Platycelphium	+	simple samara	1	N
Sakoanala	+	simple samara	3–4	N
Spirotropsis	+	simple samara	1(3)	N
Sweetia	+	basal-seeded samara	1	N
TEPHROSIEAE (Millettieae)				
Aganope	+?	simple samara	1(4)	N
Behaimia	+	simple samara	1	N
Craspedolobium	+	simple samara	3–5	N
Cyclolobium	?	round samara	2–3	N
Dahlstedtia	+?	simple samara	2–3	N
Dalbergiella	+	simple samara	1(2)	N
Derris	+	simple samara	1(2)	N
Kuntsleria	+	simple samara	1(3)	N
Leptoderris	+	simple samara	1(2)	N
Lonchocarpus	+	simple samara	1(4)	N
Margaritolobium	+?	simple samara	3–4	N
Millettia	+	endocarp segments	1	N
Piscidia	?	4 broad wings	1–10	N
Platycyamus	+?	simple samara	2–3	N
Xeroderris	+	simple samara	1(2)	N
THERMOPSIDEAE				
Ammopiptanthus	+	simple samara	3–8	N
Anagyris	?	simple samara	>4	N
Piptanthus	+	simple samara	3–8	N
TRIFOLIEAE				
Medicago	+	"tumbleweed" (spirally curled fruit only)	1–4	H
Trifolium	+	inflated calyx; pappus calyx; "tumbleweed" (infructescence only)	1–6	H
Trigonella	+?	round samara	1	H

THE EVOLUTION OF GENERA IN THE LEGUMINOSAE

Ernest Small[1]

Abstract

The evolution of genera begins with speciation and culminates with the establishment of a distinctive morphological gap between the group of species included in the genus and other species. The "genus problem" is substantially that extant groups of species exhibit various degrees of completion of generiation. Incongruence of phenetic, phylogenetic, and ecological criteria, the determinants of the genus, exacerbate the problem. Much literature concludes that genera are artificial conceptual aggregates without physical rationale for their existence; in fact, the genus is at least as evident a grouping as the species. It has been hypothesized widely that generiation is adaptively based, the notable morphological gap between most genera reflecting a notable corresponding shift in adaptive mode. The credos of defining genera either on a strictly monothetic or a strictly cladistic basis are questionable, taxonomic art having as much a role to play as at other levels of the taxonomic hierarchy. In the Leguminosae more than 90% of genera appear to have been distinguished by nonoverlapping characters, indicating good separation and a comparatively minor genus problem. More than 75% of characters used to delimit genera in the Leguminosae are reproductive and appear to have evolved more rapidly than vegetative characters. Insects have been the most important agents of change, the specificity of their adaptive pressures explaining in considerable measure the qualitative differences in adaptation and morphology among legume genera. About half of legume genera have no more than three species, and only about 1% have gone on to differentiate more than 300, so that generiation is essentially an evolutionary dead end for many, but the pathway to explosive evolutionary success for a select few.

I. Generiation

The evolutionary processes producing species, commonly known as speciation, have been examined extensively. Although there is disagreement on the definition of the product, species (see Small, 1979, for 70 different definitions), this has not hindered useful discussion of the process, speciation. This paper addresses generic formation in the Leguminosae, with emphasis on explanation in terms of biological causes and adaptive responses. Although the product in this case, genera, is even less susceptible of definition than the species, I believe it will be helpful to employ the analogous term *generiation* (as used, for example, by Stanley, 1975) to refer to the evolution of genera. Surprisingly, the term generiation is not widely used, for as Simpson (1961, p. 199) pointed out, it has long been believed by taxonomists that the genus is fundamentally different from higher-rank taxonomic categories and that genera are often the easiest category to recognize. This is true not only for plants, but also for animals (Stanley, 1979, chapter 4).

A reviewer of this paper concluded, as others have, that what is termed "generiation" here is merely speciation and time, and that the concept's characterization is disconcertingly circular and does not seem to uniquely distinguish genera from higher supraspecific taxa. As I will argue, however, considerations of adaptation and relative discontinuity lead to a concept of the genus that, like many other vague scientific concepts, deserves recognition (see Rosenberg, 1975, for a defense of vagueness in scientific language). At least a vague concept of the genus is prerequisite to an evaluation of generic evolution in the Leguminosae.

Generiation may be said to be completed once populations are sufficiently differentiated that they are considered to belong to different genera. The circularity of this characterization is no more an

[1]Biosystematics Research Centre, Agriculture Canada, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

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obstacle to discussion than it is for speciation, although obviously how taxonomists perceive the genus should be examined. If a satisfactory understanding of when generiation is completed is not easily made, neither is it easy to decide when generiation begins. It might seem that the evolution of genera should be conceptualized as populational differentiation beyond speciation, but a more reasonable approach is to include speciation within generiation (cf. Grant, 1981, p. 487). One may perhaps best begin to examine the nature of the genus by referring to the provocative observation in Linnaeus's *Philosophia Botanica* (Dictum 169 in the first edition, 1751):

> Scias Characterem non constituere Genus, sed Genus Characterem.
> Characterem fluere e Genere, non Genus e Charactere.
> Characterem non esse, ut Genus fiat, sed ut Genus noseatur.

This is usually translated (Mayr, 1969): "It is the genus that gives the characters, and not the characters that make the genus." Thus the value of characters as delimiters of the genus may only be evaluated *a posteriori*, after the genus has been recognized, and there is nothing inherent in given characters or kinds of characters that intrinsically suit them for use as generic characters.

Linnaeus exemplified his dictum by *Trifolium*, in which he included *Melilotus*. This was typical of many of Linnaeus's genera, which were often more encompassing than the genera of today. For Linnaeus, the genus was a natural (divinely created) grouping. Genera were understood to be separated by sharp discontinuities (Mayr, 1982, p. 175), although he suggested that nature was more continuous at higher categories, which were relatively artificial constructs.

In a critical analysis of the nature of genera with reference to the Gramineae, Stebbins (1956) posed two fundamental questions: "To what extent are genera artificial aggregates of species, grouped together for the convenience of taxonomists, and to what extent are they natural groupings which reflect the result of evolutionary processes?" and "Do the evolutionary principles underlying the origin of genera differ materially from those which operate in the origin of species?"

Stebbins answered the question of the naturalness of genera by stating that this varies greatly from one group of angiosperms to another. He contended that, for example, in the Gramineae diagnostic characters are scarce and artificiality of taxonomic treatment is pervasive; but in groups with abundant characters, like the Leguminosae and Compositae, this is less the case. Another complication of naturalness pointed out by Stebbins was the unevenness of gaps between groups of species, this often causing difficulties when taxonomists choose to unite diverse elements within a genus.

Regarding the evolution of genera, Stebbins concluded that generiation is a continuation of the processes that cause the origin of races and species, although later (Stebbins 1974a, p. 16) he more circumspectly pointed out that the question cannot be answered definitively with present evidence. Stebbins (1956) stated that extinction is much more important in the origin of genera than it is in the origin of species, and that as a result, in many large primitive families, among which Stebbins included the Leguminosae, authors agree on the limits of most genera, except large genera in an active state of evolution. In contrast, according to Stebbins, in phylogenetically advanced families such as the Gramineae, Orchidaceae, and Compositae, wide divergence of taxonomic opinion may exist because genera may be linked by persistent intermediate species descended from their common ancestor.

II. Adaptation and the Evolution of Generic Characters

Natural selection is widely accepted to be the principal cause of adaptive divergence, and hence of populational differentiation. In the words of Inger (1958), "Phylogeny is not synonymous with adaptation, but to a very great extent phylogeny is the history of successive adaptation." Phylogeny is also not necessarily synonymous with classification and one may inquire on the relationship of natural selection and classification. De Candolle (1813) was an early exponent of the viewpoint that classification should be based on adaptation; he felt that organs exercising more important functions

than others should play a more important role in classification. By contrast, there have been repeated suggestions that classification of organisms should be based on adaptively neutral traits (Dobzhansky, 1956). For a variety of reasons discussed by Dobzhansky, it is often difficult to interpret traits in adaptive terms, especially at the species level. Nevertheless, as explained in the following, the rectangular as opposed to gradualistic model commonly postulated for generic evolution suggests that taxonomic differences among genera are likely to be adaptive.

Currently, "macroevolution" (the evolutionary development of taxonomic units at the species level and above), is postulated to occur either gradually or punctuationally (rectangularly) in rapid episodes separated by long periods of equilibrium (see Calow, 1983, chapter 5). The clearest analysis of how adaptation may be related to macroevolution, particularly to generiation, is that of G. G. Simpson.

In Simpson's (1944) classic *Tempo and Mode in Evolution,* examples were presented showing relationships of level in the taxonomic hierarchy with a hierarchy of ecological zones. Simpson's view that increasingly greater adaptive shifts occurred historically to found the groups at increasingly higher levels of the taxonomic hierarchy has been supported by others (e.g., Hecht, 1965; Stebbins, 1974b). Simpson distinguished three modes of populational divergence differing in degree of adaptive change: speciation, phyletic evolution, and quantum evolution. Generiation falls under phyletic evolution, which Simpson described as being more strictly adaptive, more directed or sustained, and achieving a notably greater shift in adaptive mode than speciation. Simpson argued that evolution of genera and higher groups normally involves a rapid shift in adaptation. Origins of the large differences that separate supraspecific groups are explained as adaptive responses for survival under new environmental demands. Simpson's adaptive perspective of what constitutes a genus provides the rationale for the existence of the typically larger differences between genera than between species: intermediates that historically connected genera were extinguished because of absence or transience of habitats capable of sustaining them.

When closely related genera are distinguished on the basis of a single characteristic, it seems straightforward to interpret that trait as a more or less direct reflection of the adaptive difference between the taxa, representing the evolutionary response to a historical selective pressure. If the taxa are very recent, that historical pressure may still be extant, and it may be possible to analyze causally the generic divergence that occurred in rather precise terms.

When genera are large, heterogeneous, incompletely separated, or simply extremely sharply separated from allies, it is relatively difficult to interpret adaptative differences historically. If divergence has been considerable, the relatives of a genus may not be a reasonable standard of comparison. It may be that characters that evolved to meet a historical need are no longer needed for that purpose. Assessment of homologies is a constant problem (Patterson, 1982). As stressed by Janzen (1981), the nature of many traits can be understood only holistically, not as simple adaptations to single factors. Gould & Lewontin (1979) are highly critical of what they perceive to be excessive adaptive interpretation; their presentation, although unbalanced, indicates well the uncertainties of adaptive explanations for extant characters.

A special aspect bearing on the adaptive interpretation of taxonomic difference is taxonomic congruence. This is the general correlation that exists between taxonomic analyses of the same organisms based on very different kinds of characteristics (Small et al., 1982). Although exceptions are well known, there seems to be considerable taxonomic congruence for most taxonomic data. As taxa diverge, this divergence may be reflected generally in different kinds of characters, so it becomes progressively more difficult to identify the initial cause of divergence. There are, however, at least two ways of approaching the problem. First, incompletely separated genera may reflect early generic divergence and may be examined profitably. Second, based on the general understanding among systematists that the evolutionary processes producing genera are simply an extension of the processes producing species, it follows that the origin of character differentiation at the species level may be examined to show how the generic differences arrive initially.

TABLE 1. Characters used to key genera of Leguminosae in Polhill & Raven (1981).

	Numbers of characters				
Character class	Caesalpinioideae	Mimosoideae	Papilionoideae	Totals	Relative %
Life form, longevity (tree, shrub, herb, vine; annual, perennial)	3	2	32	37	2.8
Underground perennation	0	0	1	1	0.0
Stem, branch	2	1	8	11	0.8
Leaves	73	14	158	245	18.3
General vesture	7	3	10	20	1.5
Inflorescence (including pedicel, bracts, disc, receptacle)	38	18	113	169	12.6
Flower: androecium	61	10	66	137	10.2
gynoecium	20	2	60	82	6.1
corolla	51	5	112	168	12.5
calyx	21	0	69	90	6.7
other	11	4	24	39	2.9
Fruit	57	40	190	287	21.4
Seed	13	14	27	54	4.0
Totals	357	113	870	1,340	99.8

III. Generic Characters in the Leguminosae

One of the achievements of the First International Legume Conference was a multiauthored taxonomic synopsis of the tribes of the Leguminosae (Polhill & Raven, 1981). Although generic characters were not identified as such, keys for almost all of the genera were presented and these allow appreciation of the kinds of characters that taxonomists of the Leguminosae have considered capable of separating the genera. For this paper, a survey of the characters used in the generic keys in Polhill & Raven (1981) was carried out (Table 1). In order to appreciate as closely as possible characters considered appropriate to the genus level as opposed to higher and lower levels, keys to tribes and subtribal groups of genera, as well as keys to subgroupings within genera, were not examined. In all cases, characters were compiled only if (at least in my opinion) they were contrasted clearly in both legs of the key and the individual characters of character suites were logically independent. Of course, there is some redundancy in longer keys, but no effort was made to remove this. For this exercise, no attempt was made to distinguish characters that separated genera cleanly from those that were useful only in combination. This problem is addressed later. Geographical criteria were not included, so that only morphological generic characters were addressed. In the category including inflorescence characters (Table 1), features of pedicel, bracts, and receptacle were included; this grouping is made up of tissues that are directly supportive of the flower and fruit and thus are conceptually midway between vegetative and reproductive parts of the plant (although they are included as a reproductive component in the comparison of reproductive and vegetative characters).

Strictly, the present analysis is an evaluation of the characters used by a broad spectrum of taxonomists of the Leguminosae in constructing generic keys. The extent to which these characters reflect generic evolutionary affinities is debatable. Characters most useful for identification are not necessarily the best for indicating relationship, and in the construction of so-called artificial keys, characters are chosen for their utility as discriminators. Computer-derived keys are notoriously artificial. Floristic keys are often very artificial because they deal with small samples of the world's taxa, increasing the likelihood that characters of no phylogenetic value will be of discriminating value in the area covered by the flora. This is less likely in comprehensive monographic treatments.

such as those addressed here. In short keys, such as the majority examined for this paper, monographers probably have tended to avoid using artificial characters. For example, in C. C. Heyn's (1981) key to the Trifolieae, a group in which I also specialize, all of the characters used strongly reflect phylogenetic relationships of the genera.

There are obvious differences in the sources of characters that legume taxonomists have used to separate genera (Table 1). Of the 1,340 characters compiled from the keys, only one dealt with a feature of underground perennation. Roots are relatively inaccessible, are not normally observable without excavation, and are traditionally poorly collected; these considerations probably have contributed to the paucity of underground generic characters. However, as seen in Table 1, stem and branch characters contributed only 0.8% to the total, and life form only 2.8%. Clearly, the vegetative mass of Leguminosae contributed little in the way of generic characters, with the exception of the leaves, which constituted 18.3% of the total. By contrast, the reproductive component of the plants made up 76.6% of the generic characters. Fruit and seed characters collectively made up 25.4% of the total, and features of the flower 38.4%. As Table 1 indicates, all of the components of the flower provided generic characters. That more than three quarters of the compiled generic characters of the Leguminosae involve reproductive tissues suggests that evolution at the genus level in the Leguminosae has involved differential rates of evolution of vegetative and reproductive components. This is consistent with Stebbins's observation (1974a) that in the origin of higher categories, reproductive characteristics are more important than adaptations of vegetative organs. Another consistency with evolutionary theory is illustrated by the fact that all of the parts of the flower furnish appreciable generic characters (Table 1); thus the reproductive function has evolved in such a way as to maintain integration of organization, perhaps at a level comparable to that of higher animals (Stebbins, 1974a; p. 39).

Tutin (1956) pointed out that using macroscopic morphological characters of reproductive parts is traditional in vascular plant taxonomy, and that there is reason to suppose that vegetative characters, if sought more vigorously, would probably be of greater use. Although vegetative characters of generic importance may not have been sought as diligently as reproductive characters, this may not represent taxonomic negligence. The search for four-leafleted clovers, indeed of four-leafleted Trifolieae, shows how highly constrained some vegetative characters are.

Some evidence we have found for the relationships of species distribution in relation to their morphological affinities also suggests that reproductive characters evolve more rapidly than vegetative characters. The tendency for related taxa to be distributed near each other is well known for all forms of life (see Cain's, 1944 discussion of Jordon's Law). This is a consequence of related taxa diverging from a given locality. Biogeographical contiguity of taxa is obscured or obliterated during divergence, migration, extinction, and evolution of the landscape. We attempted to test the hypothesis that as geographical distance between closely related species increased during speciation, morphological similarity decreased. We demonstrated that this was the case for *Medicago*, but only for the vegetative characters (Lefkovitch, 1984, 1985; Small & Lefkovitch, 1986). This may reflect the slower evolution of vegetative characters in comparison with reproductive characters. That is, as the species of *Medicago* have diverged, related species have retained sufficient vegetative similarity to reflect a statistically significant level of association between decreasing morphological similarity and increasing geographical distance, but because of the greater rate of evolution of reproductive characteristics, the association of reproductive characters and geographical distance is no longer detectable.

IV. The Intergeneric Gap as a Theoretical Criterion

In summarizing the literature on generic concepts, Crisci (1974) stated (cf. Cain, 1956): "Definition of any taxonomic category is very difficult, but probably no taxonomic category causes as much difficulty as the genus. Since there is no operational definition available, one is forced to

adopt a pragmatic definition: the genus is the lowest higher category and the lowest established strictly by comparative data. . . . It is separated from other taxa of the same rank (other genera) by a decided gap." Mayr (1969) stated, "The ideal genus is well separated by a gap, is of the proper size, is internally homogeneous, and fills a distinctive adaptive zone." However one chooses to characterize the genus, the most important attribute is the intergeneric gap, although not all taxonomists agree (e.g., Michener, 1957). It is widely accepted that the size of the gap is commonly in inverse ratio to the size of the genera; the larger the genera the smaller the requisite gap for generic recognition (Davis & Heywood, 1963, p. 84; Mayr, 1969, pp. 92, 403, 404).

Intergeneric gaps may be incomplete because generiation, like speciation, manifests patterns of various degrees of completion. The terms "good" and "bad" are well entrenched as labels of incompletely separated taxa, although value-free words would be preferable. In any event, as discussed in the following, there is theoretical disagreement about how to treat taxonomically incompletely diverged groupings of species. Intergeneric hybridization, a principal cause of obfuscation of intergeneric gaps in other plant families, does not seem to complicate generic evolution in the Leguminosae (McComb, 1975; Löve, 1963).

At present, the comparative wisdom of a strictly phenetic approach to defining biological groups and one that espouses that taxa be defined on a phylogenetic (sensu Wiley, 1981; c.f. McNeill, 1982) basis, is open to debate. It is important to note, however, that a phylogenetic characterization of what constitutes a genus (or indeed any other category of groups), may be more stringent than that required by a phenetic characterization. As discussed by Wiley (1981, pp. 79-80), phylogeneticists usually recognize as "natural" only those groups that are diagnostically monothetic (reversals and losses may confuse interpretation), whereas pheneticists commonly accept as natural those groups that are fully polythetic (McNeill, 1982, and references therein, one of which adopts a contrary viewpoint). In monothetic sets all of the components (subsets) share all of the characters in question. In polythetic sets a substantial proportion but not all of the characters occur in each component, and each component has a substantial proportion but not all of the characters. (All components may share some of the characters; c.f. "monotypic concepts" and "polytypic concepts" in Beckner, 1959.) In the following, polythetic genera will be understood to be groups in which no discriminating character is possessed by all of the species in the genus (i.e., polythetic will be understood as *fully polythetic*), and monothetic genera will be understood to be comprised of species all of which share at least one discriminating diagnostic character (this is termed here minimally or diagnostically monothetic). It does not follow that phylogeneticists will reject as genera groups that are apparently fully polythetic, but certainly what appear to be incompletely separated genera would be viewed with suspicion. As a pheneticist, I am content to accept some fully polythetic groups as natural groups, including groups at the genus category.

If one can't separate genera cleanly, it is improbable that their separation occurred by rapid evolution as interpreted by Simpson (1944), although the possibility can't usually be excluded that lineages that previously separated rapidly, have since converged. If nearest genera are diagnostically monothetically separable, it is more likely that they diverged by rapid evolution sensu Simpson than if only fully polythetically separable. It doesn't necessarily follow that because there is a clear morphological gap between neighboring genera that they diverged quickly, as Simpson holds is the general case for phyletic evolution, but the absence of intermediates is suggestive that the adaptive zone separating the genera has been a kind of no-man's-land, in which intermediates were incapable of long-term survival. Later some examples will be presented.

V. The Intergeneric Gap in the Leguminosae

As an indication of how "good" the genera of Leguminosae are, the generic keys in Polhill & Raven (1981) were examined to assess the proportion of genera that are apparently minimally monothetic (i.e., keyed out on the basis of at least one nonoverlapping characteristic). If a genus

TABLE 2. Size (number of species) and sharpness (minimally monothetic or fully polythetic for diagnostic characters) of genera of Leguminosae as reflected by treatments in Polhill & Raven (1981). Excludes Mirbelieae for which a key was not provided in Polhill & Raven.

Parameter	Caesalpinioideae	Mimosoideae	Papilionoideae	Leguminosae	Relative %
Number of minimally monothetic genera	144	47	386	577	90.9
Mean number of species in minimally monothetic genera	12.1	16.6	26.3	22.0	—
Number of fully polythetic genera	9	13	36	58	9.1
Mean number of species in fully polythetic genera	27.6	170.9	38.6	67.0	—
Number of monospecific genera	55	13	108	176	27.7
Number of genera	153	60	422	635	—
Number of species	1,996	3,001	11,500	16,497	—

was not sufficiently understood so that a number of species could be estimated in a given treatment, data for it were not compiled. Where a key was apparently natural, separation of genera was accepted as diagnostically monothetic if only the final legs of the key separated them cleanly, although earlier legs required a combination of characters to achieve separation. Mistakes in treatments were not corrected. The number of species in the diagnostically monothetic and fully polythetic genera, and the number of monotypic genera, were evaluated also. Estimates of number of species sometimes involved a range, in which case the mean was used, or a figure such as "100 spp.," in which case the number given was adopted.

This analysis may be misleading in several respects: individual monographers may have represented characters and hence genera as more definitive than they really are; artificial keys, constructed primarily for identification rather than to contrast closely related taxa, may have represented genera as less definitive than they really are. Furthermore, many of the genera were described tentatively, with the indication that they required additional study and revision. Nevertheless, this exercise indicates generally the separation of currently accepted genera of Leguminosae. The results are given in Table 2.

The proportion of genera in the Leguminosae that have been cleanly separated by at least one character is high—over 90% (Table 2). This figure is at least comparable to the proportion of "good species" commonly encountered and verifies the ability of taxonomists to perceive genera with relative ease, at least in the Leguminosae. The gaps separating the genera of Leguminosae are characteristically well developed.

As expected, the mean number of species in the polythetic genera is larger than in the monothetic genera (64 vs. 22), in accord with the principle that the distinctiveness of genera is inversely proportional to their size. The proportion of genera of Leguminosae that are monospecific is large (ca. 28%). In adaptive terms, this may mean that generiation is presently occurring rapidly, or more likely that generiation into narrow adaptive zones incapable of supporting appreciable differentiation occurred in the past.

VI. The Agents of Change in the Leguminosae

In *Flowering Plants—Evolution above the Species Level* Stebbins (1974a) devoted a chapter each to what he perceived to be the three major parts of the plant in which adaptations of supraspecific significance occurred. These chapters respectively dealt with vegetative adaptations for survival, adaptations for cross-pollination, and adaptations for dispersal and establishment of seeds. As

pointed out earlier, more than three quarters of generic characters in the Leguminosae are reproductive. A simple reason for the predominance of macroscopic reproductive characters is probably that much of vegetative adaptation is physiological, hence cryptic. Another reason is likely that because the vegetative organs are externally structurally very simple (leaves, branches, roots) and very conservative despite their plasticity, major alterations are likely to be deleterious. Many of the vegetative generic characters in the Leguminosae, and indeed in vascular plants in general, are more or less obviously adaptively related to meteorological conditions (edaphic or atmospheric supply of water, insolation, temperature extremes, length of growing season) and to animal associates, mostly insect herbivores and vertebrate grazers. The adaptations against animals usually are of a generalized sort (spines, glands) that seem to serve as protection against a wide range of animal species, rather than against specific species.

By contrast, reproductive adaptations in the Leguminosae are much more evidently guided by biotic agents rather than by environmental forces and often have evolved in association with specific animals, predominantly insects. As noted earlier, almost 40% of generic characters in the Leguminosae come from the flowers, exclusive of the remainder of the inflorescence. The predominance of insect pollination, and the diversity of specific floral-pollinator relationships (Leppik, 1966), make it evident that pollinators have been the chief biological agent responsible for generiation in the Leguminosae. As pointed out by Arroyo (1981, p. 736), "The very success of the Leguminosae is reflected in the utilisation of bees as pollinators." Fruit characters, the next most important class of generic characters in the Leguminosae, are important adaptively as a means of protection and of seed dispersal. Once again, insects are an important generic evolutionary stimulus by means of feeding pressures (e.g., Johnson, 1981). Seeds per se provided relatively few generic characters, perhaps because the characters are usually microscopic, hence relatively difficult to evaluate and compare.

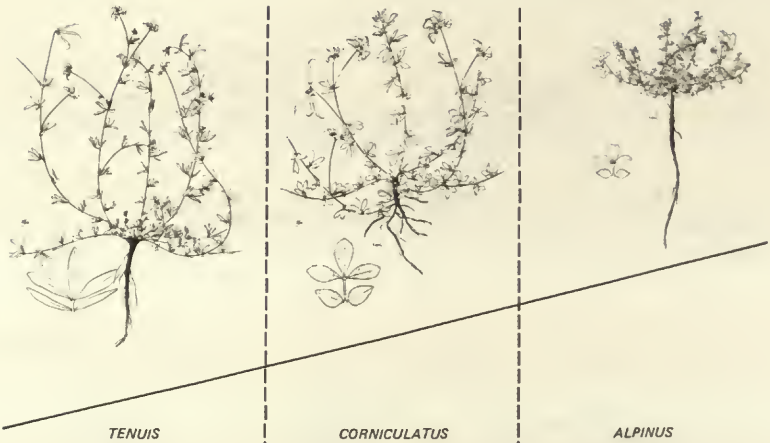
Insects are the predominant *raison d'être* for legume genera. This is indicated by the fact that many of the generic characters are adaptive to insects in general and by the specificity of the associations. It is much more likely that generiation was initiated by a specific, strong sustained selection pressure from a given insect species than from the generalized, weaker pressures of meteorological, edaphic, and other forces.

The predominant importance to vascular plants of insects as agents of change compared to other groups of organisms—other animals, fungi, bacteria, viruses, and other plants—is perhaps obvious, but bears pointing out. Even more so than meteorological factors, adaptation to fungi, bacteria, and viruses is physiological, not macroscopic. Other animals are occasionally important as dispersal vectors, pollinating agents, and grazers, but for the most part they have not developed specific biological associations with given plant species. Different species of plants are of course generally important in each other's evolution as competitors, limiting the number of niches that can be occupied, and stimulating niche differentiation. In this sense, an extant ancestor or sibling provides the most critical biological pressure for an evolutionary novelty to generiate.

VII. Examples of Early Stages of Generiation (i.e., speciation) in Which Character Differentiation is Adaptive

Ecoclinal variation in the *Lotus corniculatus* complex

The name *Lotus corniculatus* L. is variously applied by taxonomists to some or all of a polymorphic group of Old World intergrading variants. Particularly contentious are three taxa (Fig. 1), sometimes recognized as species, sometimes as varieties: *L. corniculatus* s. str. (*L. corniculatus* var. *corniculatus*), *L. tenuis* Waldst. & Kit. ex Willd. (*L. corniculatus* var. *tenuifolius* L.), and *L. alpinus* (Ser. in DC.) Schleicher ex Ramond (*L. corniculatus* var. *alpinus* Ser.) (Heyn, 1970).



TAXON	*TENUIS*	*CORNICULATUS*	*ALPINUS*
MEAN ALTITUDE	992	1553	1924
CHROMOSOME NO. (2n)	12	24	12,24

FIGURE 1. Variation in the *Lotus corniculatus* complex in Turkey in relation to altitude.

These taxa were studied intensively during a collecting expedition in Turkey (Small et al., 1984). Because the taxa usually have been described as tending to occupy different altitudes, an attempt was made to see how the characteristics traditionally used to define them were related to altitude. It was found that three groupings could be discerned altitudinally on the basis of chromosome number, lowland forms being almost completely diploid ($2n = 12$), middle altitude forms almost completely tetraploid, and high-altitude plants sometimes diploid, sometimes tetraploid (Fig. 1).

Despite this cytological basis for the recognition of three distinct taxa, the characters that traditionally have been used for diagnosis were found to vary in a more or less continuous or clinal fashion with altitude. The possible adaptive basis of the continuous characters is discussed in Small et al. (1984). Adaptive differentiation along environmental gradients is well known, probably accounting for the speciation pattern in most plants. The example is presented as a reminder of the importance of gradualistic character differentiation, which typically is expressed in a quantitatively continuous fashion.

DISRUPTIVE VARIATION IN THE *MEDICAGO SATIVA* COMPLEX

This example is also at the level of speciation, but illustrates the adaptive evolution of qualitatively rather than quantitatively different adaptive taxonomic characters.

Medicago sativa L. is extremely polymorphic, including as many as 100 variants designated as species by various taxonomists (Small & Brookes, 1984a). I have concluded that there are three important determinants of variation (Small, 1985a): adaptation to insects (see below), polyploidy (Small, 1983, 1985c; Small & Bauchan, 1984), and hybridization (Small, 1984; Small & Brookes, 1984a). The adaptations to insects are of interest in that they illustrate characters that have been used widely in *M. sativa* as the principal basis of classification, without appreciable understanding of their adaptive basis. In Figure 2, the characters that are considered to be derived are identified, along with the three groups of insects that appear to have provided the critical pressures for disruptive evolution.

Most species of *Medicago* are yellow-flowered. *Medicago sativa* is polymorphic for flower color, subsp. *falcata* (L.) Arcangeli and subsp. *glomerata* (Balbis) Rouy being yellow-flowered,

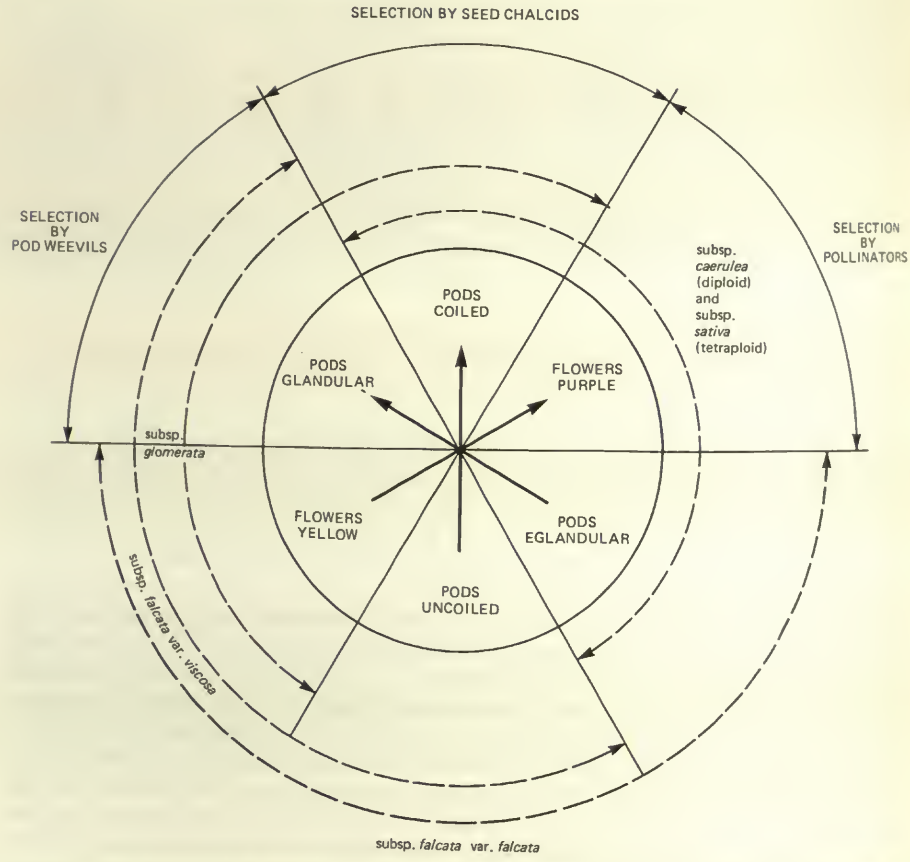


FIGURE 2. Variation in the *Medicago sativa* complex. Heavy arrowheads point to adaptive characters and the insects that appear to have provided the critical selective pressures. Broken lines designate taxa.

with violet flowers found in subspecies *sativa* and *caerulea* (Lessing ex Ledebour) Schmalhausen. *Medicago* is pollinated particularly by several genera of bees (Tassei et al., 1978; Ivanochko, 1979; Guy & Dattée, 1982), and there are clear differences in the attractiveness to pollinators of the two flower colors (Goplen & Brandt, 1975), although the species responsible for the divergence in color are not known.

Glandular-podded forms of *Medicago*, *M. sativa* subsp. *glomerata* and subsp. *falcata* var. *viscosa* (Reichenb.) Pasp. are found in the mountainous regions of the Old World, particularly in the Caucasus and adjacent regions of the U.S.S.R., but also in southern Europe and northern Africa (Small, 1986). The fruits of these taxa are covered with gland-tipped trichomes that appear to protect the seeds against the seed-eating weevil *Tychius* (Coleoptera, Curculionidae). For several reasons discussed in Small (1985b), it appears that *Tychius* was responsible for the localized evolution of fruit glands.

Of the subspecies of *Medicago*, only subsp. *falcata* possesses uncoiled fruit. Fruit coiling is highly defensive against the seed chalcid *Bruchophagus roddi* Gussakovsky (Hymenoptera, Eurytomidae) (Small & Brookes, 1982, 1984c). This insect is the most devastating pest of commercial alfalfa used to grow seed, annually accounting for millions of dollars in damage, and often virtually destroying seed crops. No effective control measure has been known against the insect, but the

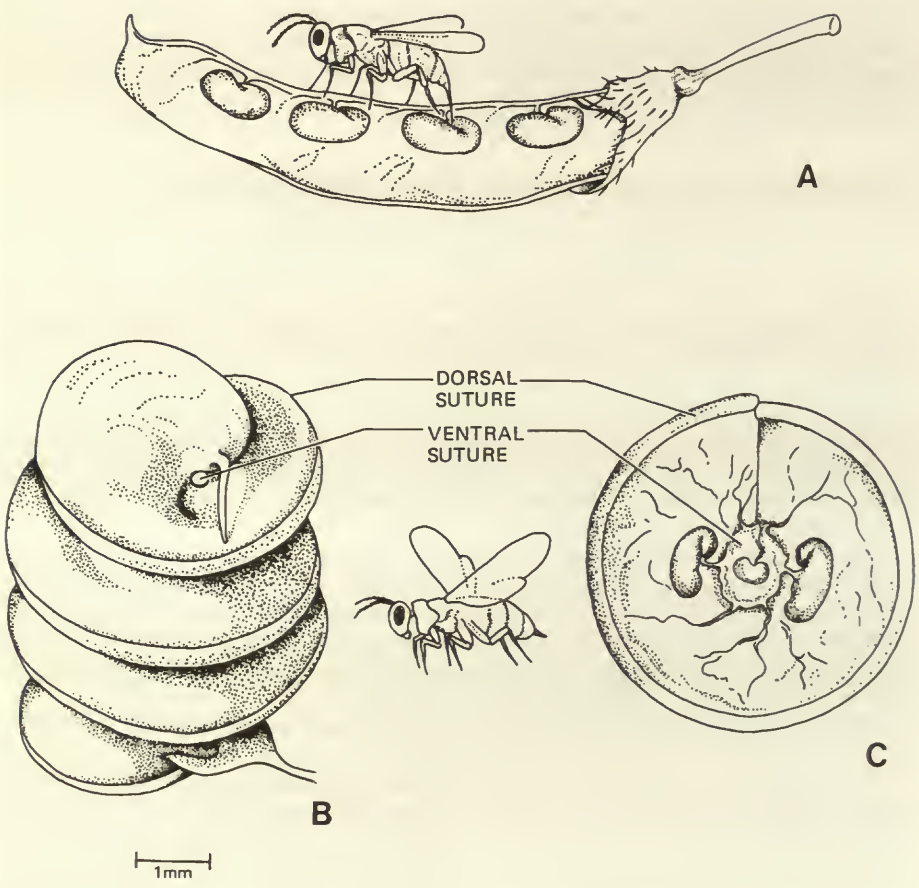


FIGURE 3. How coiling in *Medicago sativa* is defensive against seed chalcids *(Bruchophagus roddi).*—A. Seed chalcid ovipositing into seed of uncoiled fruit of subsp. *falcata,* near ventral suture.—B. Coiled fruit of subsp. *sativa,* exposing winding dorsal suture to chalcid, while protecting ventral suture within axis of coil.—C. Cross section of young coiled fruit, showing how young seeds are remote from dorsal suture, and hence out of range of chalcid's short ovipositor.

observation that the coiling of fruit is protective allows plant breeders a means of creating resistant cultivars. *Bruchophagus* damages seeds by ovipositing through the fruit wall directly into young seeds. However, we found that the insect's ovipositor is too short to reach the seeds in coiled fruits because the seeds are too remote from the ventral (placental) suture, which constitutes the axis of the coil (Fig. 3). Fruit coiling has been considered to be a generic character for the genus *Medicago.* This example illustrates how a qualitative character capable of generic separation may sometimes arise and be perfected at an infraspecific level.

All of the important qualitative characters in the *Medicago sativa* complex (flower color, fruit glandularity, fruit coiling) are adaptive responses to particular insects. It has been asserted that subspecies and species are usually defined in quantitative terms, but genera and higher groups are often defined in qualitative terms (see Simpson, 1943). The *M. sativa* complex does not conform to this belief, which in any event is known to have many exceptions and to be partly semantic (since the difference between qualitative and quantitative characters is partly one of formulation). How-

ever, qualitative differences are particularly apparent among "good" genera. Examples of clear qualitative differences that have been proposed as the basis for generic recognition are presented below. Nevertheless, even with clear qualitative discontinuities, the essential continuity of evolution makes definition of genera problematical.

VIII. Generiation in Tribe Trifolieae, Subtribe Trigonellinae

Four or five closely related genera are currently placed in Trifolieae subtribe Trigonellinae: *Medicago, Trigonella, Factorovskya,* and *Melilotus,* and sometimes *Rhodusia.* There has been disagreement for centuries concerning generic circumscription in the subtribe Trigonellinae, and still additional genera have been recognized by splitting (Small, 1981). My solution, recognizing *Medicago* as a comprehensive genus of about 82 species, is detailed elsewhere (Small et al., 1987; Small, 1987a). The following presentation examines the adaptive basis of the characters employed to recognize genera, at least for those that have been proposed with some taxonomic insight. These competing circumscriptions are all based essentially on perceivable gaps and are defensible in their own rights. However, when the evolution of the generic characters in the Trigonellinae is arranged in an evolutionary sequence and interpreted adaptively, several aspects of the genus problem, and its solution in *Medicago,* become apparent.

An interpretation of the phylogeny of the Trifolieae, based on my research over the last several years, is given in Figure 4. The primary concern is evolution of groupings that have been represented by some taxonomists in the past as belonging to *Medicago.* Figure 4 shows six ordered stages of evolution of the characters that have been used to circumscribe *Medicago.* Stages 4, 3, 2, and 1 have been progressively less widely accepted in the literature as constituting *Medicago.* (That such an ordering can be made is not apparent from the literature.)

In the first stage of the evolution of *Medicago,* a radical shift in mode of pollination occurred, from a mechanism allowing repeated transfer of pollen to visiting pollinators, to one in which an irreversible explosive transfer occurs. At least 12 morphological features distinguish the flowers of species possessing this mechanism (all groups following evolutionary event 1 in Fig. 4) from all other Trifolieae (Small et al., 1981; Small & Brookes, 1983, 1984b). We have interpreted all of these differences in adaptive terms (Small et al., 1987; Small, 1987a). It is clear that there has been functional correlation in the changes that have co-occurred in all parts of the flower, adapting the flower to a radically different way of life. In comparison with *Trigonella* s. str., *Melilotus,* and *Trifolium,* the pollination mechanism of *Medicago* seems to be relatively efficient in transferring a given mass of pollen to an ovule, i.e., less energy is required. This change could be termed an evolutionary advance (Small, 1987b).

In the second stage of the evolution of *Medicago* cotyledonary pulvini were lost. The phenomenon may be associated with fruit dehiscence. Most species preceding evolutionary event 2 have dehiscent fruit that is generally straight and cylindrical. Note in Figure 4 that following (concomitant, really) with the loss of cotyledonary pulvini two lineages emerge, one with flat fruits, the other with coiled fruits. Fruits that are flattened seem adapted to wind dispersal (Heyn, 1968), and seeds need to be retained for at least some period within the fruit. Fruits that are coiled are usually indehiscent or tardily dehiscent (except two of the three species placed here in section *Medicago*). It often happens in indehiscent-fruited *Medicago* that the seeds germinate before the pericarp has weathered, rotted, or split appreciably from various environmental damages. In order to escape from the fruit the shoot of the seedling must emerge through a crack in the pericarp. In this situation, if the cotyledons possess pulvini they may flex at this point, preventing egress of the shoot of the seedling from the fruit (Fig. 5). Viewed in this light, the evolutionary loss of pulvini may be a by-product of fruit specialization.

In the third stage of *Medicago* evolution (Fig. 4) fruit coiling has developed. Earlier it was discussed how fruit coiling has undergone disruptive evolution in *M. sativa,* the seeds in subspecies

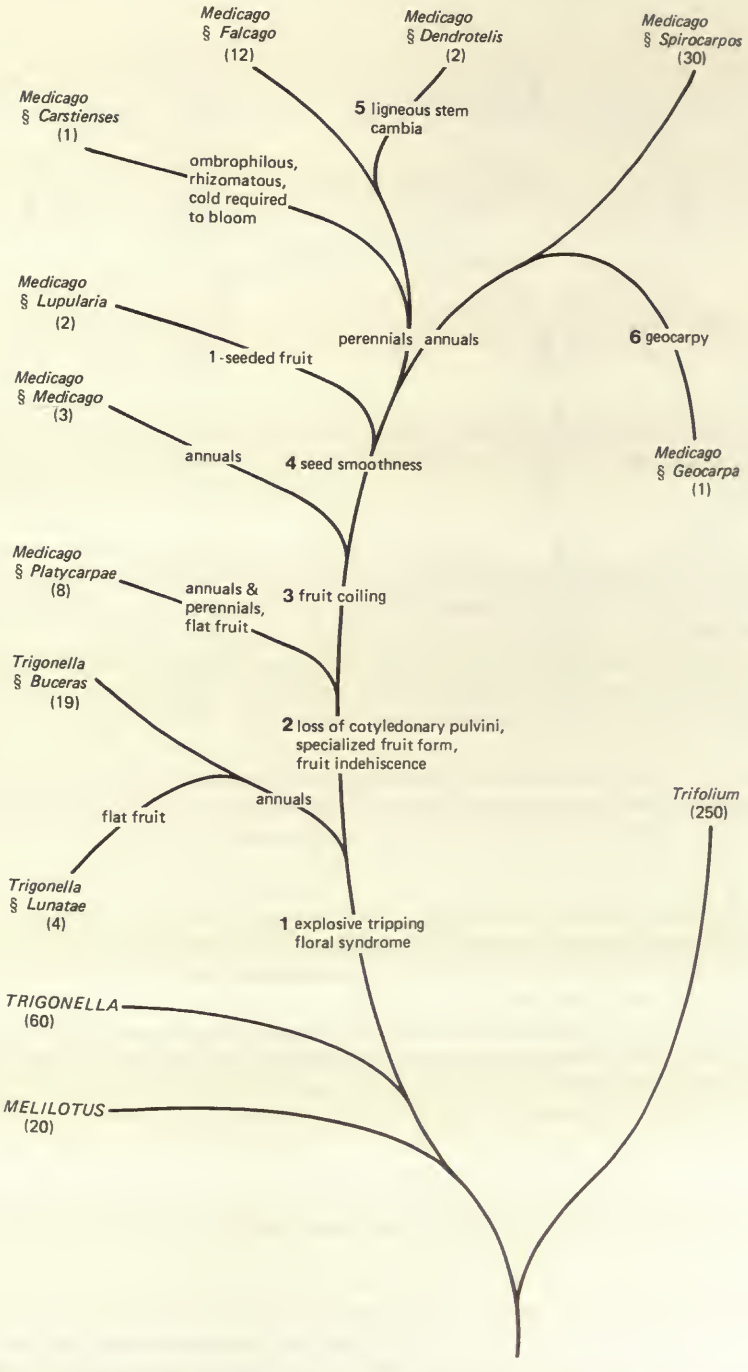


FIGURE 4. Phylogenetic tree of tribe Trifolieae. Numbers of species in given groups are shown in parentheses. Six critical stages of character evolution, which may be used to circumscribe *Medicago* more or less comprehensively, are shown.

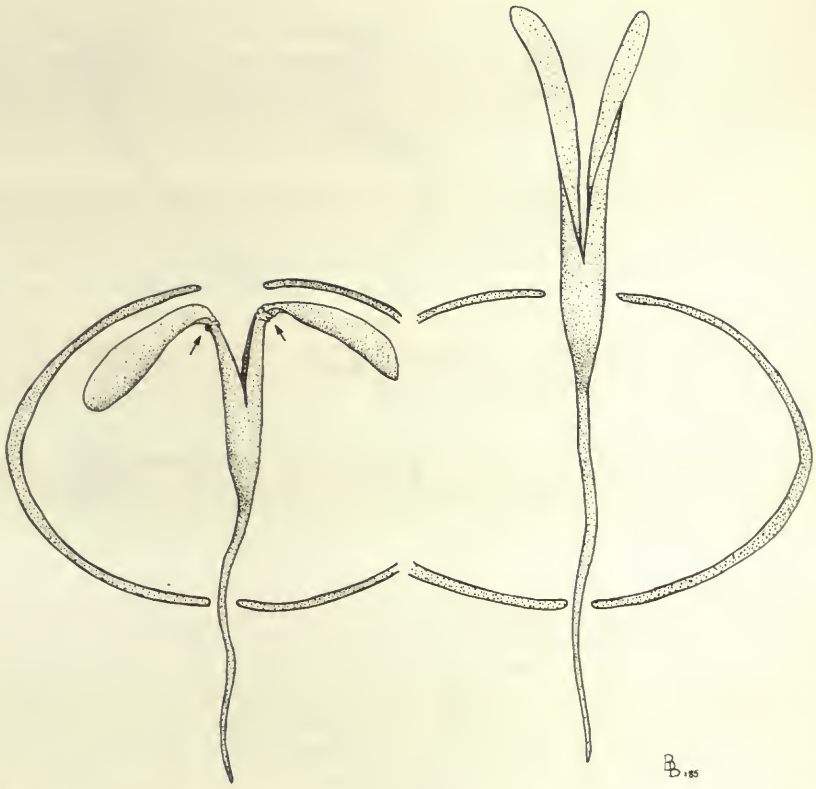


FIGURE 5. A rationale for the evolutionary loss of cotyledonary pulvini in indehiscent fruits. Left, cotyledons flexed at pulvini (arrows) are unable to escape through hole and remain trapped inside fruit. Right, cotyledons lacking pulvini are sufficiently straight to be able to escape fruit through a small hole.

with coiled fruits being highly protected against seed chalcids. Whether seed chalcids were responsible for the original evolution of fruit coiling is unclear. It is evident, however, that fruit coiling is highly adaptive and probably arose in *Medicago* as an adaptation.

In the fourth stage of evolution of *Medicago* (Fig. 4) seeds have become smooth. In previous groups, many of the species have bumpy seed surfaces because of some sort of a pattern of papillae or striae. The various kinds of bumps and ridges represent extra tissue produced by the plant as the result of energy expenditure and presumably are present because they are adaptive. I believe that seed roughness provides protection for the seed, perhaps against herbivores, or perhaps is present to resist abrasion during dissemination, or to increase friction to help the seed remain in place in the substrate (Fig. 6). It is surely not by chance alone that almost all of the species following evolutionary event 2 that have indehiscent or tardily dehiscent fruits have seeds lacking roughness. Obviously, seed roughness is useful in the more primitive species of subtribe Trigonellinae possessing dehiscent fruits. As with evolutionary event 2 (loss of cotyledonary pulvini), the evolutionary loss of roughness of the seed surface is probably a by-product of fruit specialization.

The fifth stage of evolution shown in Figure 4 produced section *Dendrotelis* with two species. These arguably constitute the separate genus *Rhodusia* Vass. The species are large shrubs (up to 3 m), much taller than other Trifolieae. With the exception of *M. marina*, these are the only *Medicago* with perennial aboveground stems. (Other perennials produce annual aboveground stems.) Although there are many lignescent perennial shrubby forms in the Trifolieae, the species of section

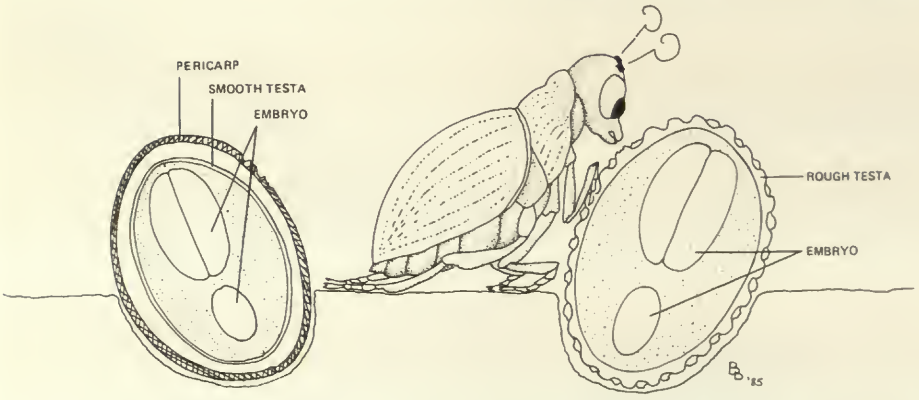


FIGURE 6. A rationale for the evolutionary loss of seed roughness in indehiscent fruits. Left, smooth seed retained in indehiscent fruit is protected by pericarp. Right, free seed may use raised surface to fend off herbivores and increase friction against substrate.

Dendrotelis are the only ones with woody aboveground stems, with one cambium producing abundant wood, and another phellogen. As has often been done for trees, one might be tempted to interpret these woody species as relics of ancient derivation. However, they possess too many specializations for this interpretation (i.e., 1–4 inclusive in Fig. 4), and their woodiness is absent in relatives. As a result of their vigor, plants of section *Dendrotelis* show extreme development of size characters (e.g., they have the largest flowers in *Medicago*), and there is a large gap in quantitative expression of many characters separating these *Dendrotelis* species from their relatives. Whether this constitutes a reasonable basis for generic segregation is debatable.

The sixth notable change in adaptive mode is the evolution of geocarpy (5 in Fig. 4). We recently transferred the enigmatic *Factorovskya aschersoniana* (Urb.) Eig. to *Medicago* as *M. hypogaea* E. Small (Small & Brookes, 1984b) after we discovered that it has all of the characteristics (1–4 in Fig. 4) that have been used to define *Medicago*. As with other geocarpic species, it appeared that the taxonomic distance from relatives with aerial fruits had been overestimated because of profound differences inherent in geocarpy (Small & Brookes, 1984b). Notably, there are two geocarpic species in *Trifolium* that were not segregated from that genus on the basis of geocarpy alone. Geocarpy is an extreme adaptation; obligately geocarpic species have entered a radically new adaptive zone. It seems certain that this particular species is an evolutionary dead end. Despite the flowers having all of the outcrossing morphological adaptations of true *Medicago*, the flowers are the smallest in the genus, invariably selfing. The fact that *Factorovskya* was not suspected to be a *Medicago* until a century after its discovery indicates that it is different, and that there is an arguable case for segregating it as a separate genus.

Accepting that *Medicago hypogaea (Factorovskya)* and section *Rhodusia* deserve separate generic recognition poses a philosophical problem if one also accepts the phylogeny of these species, shown in Figure 4, as derivatives of true *Medicago*. In Figure 4, any one of the linearly sequential evolutionary changes are synapomorphies, characterizing the subsequent lineage. By the cladistic school of thought, they may legitimately be used to define the genus. Having done so, all subsequent derivatives fall within that taxon. The concomitant use of a subsequently derived character, such as the apomorphies that define *Factorovskya* and *Rhodusia*, turns *Medicago* into a paraphyletic group (i.e., one possessing a common ancestor, but not all extant derivatives of that ancestor; c.f. Funk, 1985). Although this cladistic limitation is in accord with my circumscription of *Medicago* as inclusive of all species derived after synapomorphy 1, most taxonomists probably would not

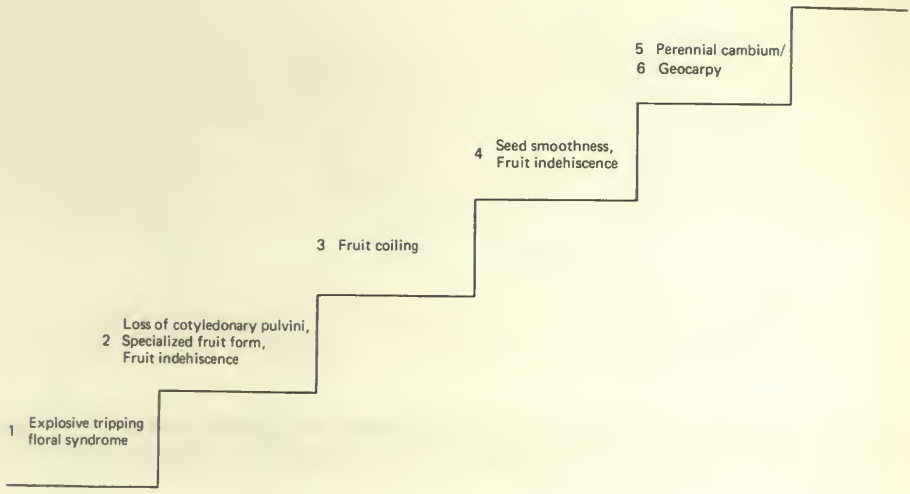


FIGURE 7. Rectangular (punctuated) evolution in *Medicago* sens. lat. (cf. Fig. 4).

accept a purely cladogenetic criterion for defining a taxon, when, as with the derivation of *Factorovskya*, considerable anagenetic change has occurred.

In Figure 4, variation in *Medicago* sens. lat. is categorized into ten sections (for further information, see Small, 1987a). Sections *Geocarpa (M. hypogaea)* and *Dendrotelis* are markedly different from other *Medicago* and have been proposed to constitute different genera. By contrast, section *Carstienses* makes up the species *M. carstiensis*, which is not as evidently different as to have stimulated generic segregation. In fact, it is a very different kind of organism physiologically (Lesins & Lesins, 1979). It is possibly as genetically differentiated from other *Medicago* as are sections *Dendrotelis* and *Geocarpa*, but given the morphological bias of higher plant taxonomy, a proposal to separate it generically would not be supported.

Figure 7 portrays the six major adaptive and morphological changes that have been used to circumscribe *Medicago* more or less comprehensively. In such punctuated rectangular evolution, taxonomic delimitation of genera may seem quite arbitrary. However, the steps are not as equivalent as shown. The first step, the evolution of explosive tripping, involved an extensive morphological reorganization. For this reason I favor using it to help define the genus *Medicago*.

IX. Beyond Generiation

In Figure 8, a histogram illustrates the relative percentage of species in given genera on a logarithmic scale (the information once again compiled from Polhill & Raven, 1981). That is, the middle of the range covered by each succeeding class is ten times that of the preceding class. Doubtless with further study, some of the smaller genera will prove to have been segregated unwisely, and some of the larger "umbrella genera" will prove to make up distinctive groups. It is unlikely, however, that the overall relative percentages will diverge greatly from those shown in Figure 8. The results are similar to those of Clayton (1972), who found that there generally was a logarithmic relationship between species numbers in genera and number of genera in five families, including the Leguminosae. One might characterize the four classes as illustrative of genera, from the point of view of evolutionary diversity, as dead ends (1–3 species), moderately successful (4–31 species), highly successful (32–316 species) and very highly successful (more than 316 species).

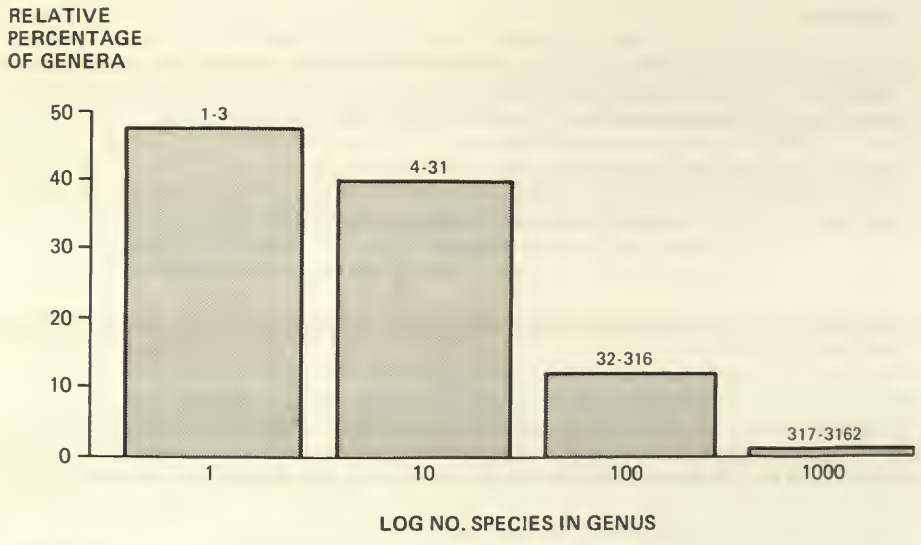


FIGURE 8. Histogram showing relative percentage of genera of Leguminosae with given numbers of species. Species numbers, obtained for the 658 genera derived from in Polhill & Raven (1981), are plotted logarithmically, with untransformed range of numbers shown on top of the histogram.

Almost half of the genera of Leguminosae have differentiated less than four species. It might be that such small genera are simply recent from a temporal viewpoint. More likely, they represent plants that have adapted to a narrow ecological niche and have very limited potential for escape from it. By contrast, although only about 1% of legume genera are successful to the point of differentiating more than 300 species, such impressive radiation suggests an evolutionary vigor that has been or will be responsible for future phyletic evolution (c.f. Stanley, 1975).

Mayr (1963) pointed out that while most species are evolutionary dead ends, those developing combinations of characteristics allowing exploitation of new ecological zones may become the progenitors of new dominant groups. If most species are evolutionary dead ends, the same can be said for most genera. It would seem fair to judge the success of a given genus in terms of future evolution by its size and diversity. Of course, we cannot know what the future holds for plant evolution. However, it is unlikely that the monotypic genera of legumes will continue to differentiate notably beyond their present status. This is not to suggest that highly isolated species are unimportant in evolution, for almost surely a small percentage of monotypic genera go on to produce families and groups of still higher categories. However, when we examine the extant range of plant variation, it is likely that the founders of future lineages will occur in large, vigorous genera, and indeed in ones that pose generic problems. Paradoxically then, good genera are probably mostly moribund, while bad (poorly defined) genera almost certainly gave rise to most of our present vascular plants and will continue to be the basis of most future plant evolution.

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References

Arroyo, M. T. K. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–769 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Beckner, M. 1959. The Biological Way of Thought. Columbia Univ. Press, New York.

Cain, A. J. 1956. The genus in evolutionary taxonomy. Syst. Zool. 5: 97–109.

Cain, S. A. 1944. Foundations of Plant Geography. Hafner, New York.

Calow, P. 1983. Evolutionary Principles. Blackie, Glasgow & London.

Candolle, A. P. de. 1813. Théorie Elémentaire de la Botanique. Déterville, Paris.

Clayton, W. D. 1972. Some aspects of the genus concept. Kew Bull. 27: 281–287.

Crisci, J. V. 1974. A numerical-taxonomic study of the subtribe Nassauviinae (Compositae, Mutisieae). J. Arnold Arb. 55: 568–610.

Davis, P. H. & V. H. Heywood. 1963. Principles of Angiosperm Taxonomy. D. Van Nostrand, Princeton, New Jersey & New York.

Dobzhansky, T. 1956. What is an adaptive trait? Amer. Naturalist 90: 337–347.

Funk, V. A. 1985. Cladistics and generic concepts in the Compositae. Taxon 34: 72–80.

Goplen, B. P. & S. A. Brandt. 1975. Alfalfa flower color associated with differential seed set by leaf-cutter bees. Agron. J. 67: 804–807.

Gould, S. J. & R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 205: 581–598.

Grant, V. 1981. Plant Speciation. 2nd edition. Columbia Univ. Press, New York.

Guy, P. & Y. Dattée. 1982. Reproductive patterns of *Medicago sativa*. Pp. 125–141 *in* E. Porceddu & G. Jenkins (editors), Seed Reproduction in Cross Pollinated Species. Balkema, Rotterdam.

Hecht, M. K. 1965. The role of natural selection and evolutionary rates in the origin of higher levels of organization. Syst. Zool. 14: 301–317.

Heyn, C. C. 1968. An evolutionary study of fruit morphology in the tribe Trigonelleae (Leguminosae). Phytomorphology 18: 54–59.

———. 1970. *Lotus*. Pp. 518–531 *in* P. H. Davis (editor), Flora of Turkey and the East Aegean Islands. Volume 3. Univ. Press, Cambridge.

———. 1981. Trifolieae (Bronn) Benth. Pp. 383–385 *in* R.M. Polhill & P.H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Inger, R. F. 1958. Comments on the definition of genera. Evolution 12: 370–384.

Ivanochko, M. 1979. Taxonomy, Biology and Alfalfa Pollinating Potential of Canadian Leaf-cutter Bees—Genus *Megachile* Latreille (Hymenoptera: Megachilidae). M.Sc. thesis, Macdonald College, McGill Univ., Montreal.

Janzen, D. H. 1981. The defenses of legumes against herbivores. Pp. 951–977 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Johnson, C. D. 1981. Seed beetle host specificity and the systematics of the Leguminosae. Pp. 995–1027 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Lefkovitch, L. P. 1984. A nonparametric method for comparing dissimilarity matrices, a general measure of biogeographical distance, and their application. Amer. Naturalist 123: 484–499.

———. 1985. Further nonparametric tests for comparing dissimilarity matrices. Math. Biosc. 73: 71–88.

Leppik, E. E. 1966. Floral evolution and pollination in the Leguminosae. Ann. Bot. Fenn. 3: 299–308.

Lesins, K. A. & I. Lesins. 1979. Genus *Medicago* (Leguminosae). A Taxogenetic Study. W. Junk, The Hague.

Linnaeus, C. 1751. Philosophia Botanica. Kiesewetter, Stockholm.

Löve, A. 1963. Cytotaxonomy and generic delimitation. Regnum Veg. 27: 45–51.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

———. 1969. Principles of Systematic Zoology. McGraw-Hill, New York & other cities.

———. 1982. The Growth of Biological Thought. Diversity, Evolution, and Inheritance. Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

McComb, J. A. 1975. Is intergeneric hybridization in the Leguminosae possible? Euphytica 24: 497–502.

McNeill, J. 1982. Phylogenetic reconstruction and phenetic taxonomy. Zool. J. Linn. Soc. 74: 337–344.

Michener, C. D. 1957. Some bases for higher categories in classification. Syst. Zool. 6: 160–173.

PATTERSON, C. 1982. Morphological characters and homology. Pp. 21–74 *in* K. A. Joysey & A. E. Friday (editors), Problems of Phylogenetic Reconstruction. Academic Press, New York.

POLHILL, R. M. & P. H. RAVEN, (editors). 1981. Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

ROSENBERG, A. 1975. The virtues of vagueness in the languages of science. Dialogue (Canadian Philosophical Review) 14: 281–305.

SIMPSON, G. G. 1943. Criteria for genera, species and subspecies in zoology and paleozoology. Ann. New York. Acad. Sci. 44: 145–178.

———. 1944. Tempo and Mode in Evolution. Columbia Univ. Press, New York.

———. 1961. Principles of Animal Taxonomy. Columbia Univ. Press, New York & London.

SMALL, E. 1979. The Species Problem in *Cannabis*—Science & Semantics. Volume 1: Science. Corpus, Toronto.

———. 1981. A numerical analysis of major groupings in *Medicago* employing traditionally used characters. Canad. J. Bot. 59: 1553–1577.

———. 1983. Pollen ploidy-prediction in the *Medicago sativa* complex. Pollen Spores 25: 305–320.

———. 1984. Hybridization in the domesticated-weed-wild complex. Pp. 195–210 *in* W. F. Grant (editor), Plant Biosystematics, Academic Press Canada, Don Mills, Ontario.

———. 1985a. Taxonomic discoveries of interest to the alfalfa agronomist. Forage Notes 28: 24–29.

———. 1985b. Insect pests and the evolution of defensive glandular trichomes in alfalfa. Canad. J. Pl. Sci. 65: 589–596.

———. 1985c. Morphological differentiation in *Medicago sativa* s.l. in relation to ploidy. Canad. J. Bot. 63: 1747–1752.

———. 1986. Taxonomy of glandular wild alfalfa (*Medicago sativa* L.). Canad. J. Bot. 64: 2125–2129.

———. 1987. Generic changes in Trifolieae subtribe Trigonellinae. Pp. 159–181. *in* C. H. Stirton (editor), Advances in Legume Systematics, Part 3. Royal Botanic Gardens, Kew.

———. 1988. Pollen-ovule patterns in tribe Trifolieae (Leguminosae). Pl. Syst. Evol. 160: 195–205.

——— & G. R. Bauchan. 1984. Chromosome numbers of the *Medicago sativa* complex in Turkey. Canad. J. Bot. 62: 749–752.

——— & B. S. Brookes. 1982. Coiling of alfalfa pods in relation to resistance against seed chalcids. Canad. J. Pl. Sci. 62: 131–135.

——— & ———. 1983. The systematic value of stigma morphology in the legume tribe Trifolieae with particular reference to *Medicago*. Canad. J. Bot. 61: 2388–2404.

——— & ———. 1984a. Taxonomic circumscription and identification in the *Medicago sativa-falcata* (alfalfa) continuum. Econ. Bot. 38: 83–96.

——— & ———. 1984b. Reduction of the geocarpic *Factorovskya* to *Medicago*. Taxon 33: 622–635.

——— & ———. 1984c. Coiling of alfalfa pods in relation to resistance against seed chalcids: additional observations. Canad. J. Pl. Sci. 64: 659–665.

——— & L. P. Lefkovitch. 1986. Relationships between morphology, geography, and interfertility in *Medicago*. Canad. J. Bot. 64: 45–52.

———, C. W. Crompton & B. S. Brookes. 1981. The taxonomic value of floral characters in tribe Trigonelleae (Leguminosae), with special reference to *Medicago*. Canad. J. Bot. 59: 1578–1598.

———, W. F. Grant & C. W. Crompton. 1984. A taxonomic study of the *Lotus corniculatus* complex in Turkey. Canad. J. Bot. 62: 1044–1053.

———, P. Lassen & B. S. Brookes. 1987. An expanded circumscription of *Medicago* (Leguminosae, Trifolieae) based on explosive flower tripping. Willdenowia 16: 425–437.

———, L. P. Lefkovitch & D. Classen. 1982. Character set incongruence in *Medicago*. Canad. J. Bot. 60: 2505–2510.

STANLEY, S. M. 1975. A theory of evolution above the species level. Proc. Nat. Acad. Sci. U.S.A. 72: 646–650.

———. 1979. Macroevolution—Pattern and Process. W. H. Freeman & Company, San Francisco.

STEBBINS, G. L., JR. 1956. Taxonomy and the evolution of genera, with special reference to the family Gramineae. Evolution 10: 235–245.

———. 1974a. Flowering Plants—evolution above the species level. Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

———. 1974b. Adaptive shifts and evolutionary novelty: a compositionist approach. Pp. 285–306 *in* F. J.

Ayala & T. Dobzhansky (editors), Studies in the Philosophy of Biology. Univ. of California Press, Berkeley, California.

Tassei, J. N., M. Picard & S. Carre. 1978. Les insectes pollinisateurs de la luzerne (*Medicago sativa* L.) en France. Apidologie 9: 175–194.

Tutin, T. G. 1956. Generic criteria in flowering plants. Watsonia 3: 317–323.

Wiley, E. O. 1981. Phylogenetics. John Wiley & Sons, New York.

Appendix I. Glossary of Important Terms.

Adaptation—a character (or a set of characters, or a general condition, or the process by which these are evolved) through which there is fitness for a particular environment.

Category—the rank or level in a hierarchic classification.

Cladistic—based on the principles of cladism, that is, ordering and ranking entirely on the basis of recency of common descent, i.e., on the basis of the most recent branching point of an inferred phylogeny, and not temporally or on degree of physical similarity.

Classification—a delimiting, ordering, and ranking of taxa.

Genus—a distinct set of one or more species, where a species is a distinct group of indistinct individuals.

Geocarpy—the production of underground fruit.

Hierarchy—the conventional ranking system used by taxonomists to indicate categorical levels of taxa.

Homology—a feature in two or more taxa that derives from the same feature in the common ancestor of these taxa.

Key—a tabulation of diagnostic characters of taxa, usually in dichotomous couplets facilitating identification.

Key, artificial—a key based on convenient and conspicuous diagnostic characters that do not indicate relationship.

Key, natural—a key based on diagnostic characters that are thought to indicate relationship.

Monothetic—referring to taxa in which each member has the defining diagnostic character(s) of the taxa.

Monotypic—referring to a taxonomic group lacking named subgroups at the immediately subordinate rank under consideration; e.g., a genus with only one species.

Phenetic—based on ordering and ranking on degree of overall similarity, not on presumed temporal or cladistic considerations.

Phylogenetic—referring to lineages of evolution as deduced by criteria less restrictive than those of cladistics and phenetics.

Polythetic—referring to taxa in which each member has a majority of a set of diagnostic characters.

Rectangular evolution—evolution in which rapid and substantial change has occurred in a lineage, immediately before and immediately after which change has been slow or nonexistent.

Synapomorphy—presence of apomorphous characters in related taxa, i.e., characters that derive from common ancestry.

Taxon (pl. taxa)—a named group of genetically related organisms.

REPRODUCTIVE CHANGES INDUCED BY DOMESTICATION

Uzi Plitmann[1] and M. E. Kislev[2]

ABSTRACT

This paper discusses the evolution of reproductive characters during the introduction of monocarpic pulses into cultivation, with an emphasis on those domesticated in the Middle East. The Middle Eastern region is rich in leguminous annuals and was one of the primary centers of their domestication. Based on archeological evidence, five phases can be discerned in the history of a common domesticate. The whole process involved adoption and change of sets of reproductive characters such as flower and fruit phenology, productivity, mating system, and several characters of seed and pod. Comparisons between cultivated species and their wild relatives reveal that the domestication of pulses, at least at the initial phases, did not dictate significant changes in the original reproductive traits pertaining to pollination and mating systems; those were preadaptive to farmers' requirements. Since the seeds and pods were principally the parts used, they have been the major targets of selection and breeding. Consequently, morpho-anatomical characters of the seed and its coat, which are associated with various biological functions and processes or with human affairs, represent syndromes of induced changes. By comparing certain physical parameters of seeds and by using indices (such as ratio of relative increase in seed weight to relative decrease of testa thickness), we could assess the trend and relative amount of change in these organs. Major crops, regardless of their antiquity, show higher indices. These sets, combined with habit, ecological demands, and demographical features rendered special legumes (*Phaseolus* spp., *Pisum sativus, Lens culinaris,* and probably *Vicia faba*) their advantage in cultivation over other potential candidates in this and other families. Legumes with deficient combinations have been abandoned or retained as secondary crops. Artificial selection in legumes followed two general courses: adoption of preadaptations and acceleration/intensification of natural trends, e.g., selfing, early pod production, more and larger seeds, retardation of dehiscence of pods; and selection of characters (mutations) opposing the natural evolutionary trend, e.g., "sweet" seed, thinner testa, simultaneous ripening, complete indehiscence. The wide variability in populations of native annual legumes and their generally rapid reaction to selection enabled the first farmers and breeders to follow both pathways.

I. Introduction

Today more than 15 species of legumes serve as staple food in various parts of the world; seven or eight species are used as major (primary) crops. Several others are consumed locally or grown as forage/fodder plants (Purseglove, 1968; Summerfield & Bunting, 1980; Allen & Allen, 1981; Duke, 1981; Jones & Davies, 1983).

The introduction of legumes into cultivation took place in and around the Middle East (6–7 species), Central and South America (5–6 species), the Far East (1–2 species), and Africa (2–3 species). Table 1 presents 15 of the most important pulses and their origins. Data on the ancestral species and their places of origin were compiled from many archaeobotanical publications and relevant biosystematic and genetic studies. The list of wild relatives, though incomplete, refers to the taxonomic relationships of the species involved. The archaeological evidence is often fragmentary or inadequate owing to dating methods and lack of proper material, or the findings could be those of gatherers, that is, prior to agriculture with its methodical cultivation.

Domestication of pulses started in the Middle East approximately 10,000 years ago, about the

[1]Department of Botany, The Hebrew University, Jerusalem, Israel.
[2]Department of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.

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TABLE 1. Major cultivated pulses and their origins. (Relevant literature included in the text.)

Species	Ancestor	Place of origin	Wild relatives	Earliest archeological evidence
Cajanus cajan	*C. cajanifolius*	India	*C. lineatus*	2nd C BC
Cicer arietinum	*C. reticulatum*	Near East (Anatolia)	*C. echinospermum*	4500–3300 BC
Glycine max	*G. soja*	Far East (Northern China)		1700–1100 BC
Lathyrus sativus		Eastern Mediterranean (Balkan)	*L. cicera* *L. marmoratus*	6000 BC
Lens culinaris	*L. orientalis*	Near East	*L. nigricans*	7300–6500 BC
Lupinus albus	*L. graecus (subsp. graecus)*	Eastern Mediterranean (Balkan)	Mediterranean lupines	2000–1000 BC
L. mutabilis	*L. mutabilis*	South America	Andean lupines	recent & pre-Columbian
Phaseolus coccineus	*P. coccineus* var. *formosus*	Mexico & Guatemala		2200 BC
P. lunatus	*P. lunatus*	South America		7000–6000 BC
P. vulgaris	*P. aborigineus*	Central & South America		7000–6000 BC
Pisum sativum	*P. syriacum* & *P. elatius*	Near East	*P. humile*	7300–6500 BC
Vicia ervilia	*V. ervilia*	Near East	*V. quadrijuga*	7500–6500 BC
V. faba		Near East	*V. galilaea*	6500 BC
		Eastern Mediterranean?	*V. narbonensis*	
Vigna mungo	*V. sublobata* (*V. radiata* var. *sublobata*)	India		1700–1400 BC
V. unguiculata	subsp. *dekindtiana*	West Africa?		

same time that cereals were domesticated (Zohary & Hopf, 1973) or perhaps before (Kislev & Bar-Yosef, 1987). This process could have taken place in South America at about the same time, 8,000–9,000 years ago; in Africa 5,000–6,000 years ago; and in the Far East about 5,000 years ago.

Thousands of plant generations could have been subjected to artificial selection or other selection pressures in the agricultural ecosystem. Accordingly, many characters of the original stocks have probably been changed. The literature (Purseglove, 1968; Baker, 1972; Pickersgill & Heiser, 1976; Smartt, 1976a, b, 1978; Simmonds, 1979; Adams & Pipoly, 1980) displays some principal categories of changes or trends of adaptations in the domestication of grain crops in general:

A trend toward annual life form usually with a shorter life cycle;
Change of habit, reduction in plant size, a trend toward erect and compact plants;
Change in time and duration of flowering and fruiting;
Physiological changes—loss of photoperiodic sensitivity, loss of seed dormancy;
Chemical changes—reduction of toxic substances, development of attractive colors and tastes;
Chromosomal repatterning and hybrid formation;
Higher inbreeding and lower variability;
Higher productivity;
Loss of dispersal mechanisms.

These categories, which may refer to cultivated food plants in general, are deduced from comparative studies of domesticates and their wild relatives, cultigens and primitive forms.

In this paper we restrict ourselves to reproductive changes induced in monocarpic pulses, with an emphasis on those domesticated in the Middle East and the Mediterranean regions. Most of the biological changes are a reflection of human needs and the outcome of human activities. By studying these changes we may gain a better idea as to how and why certain legumes, of all plants available, were domesticated and have been so widely and extensively used by humans.

II. Historical Background

A study of the nature of induced reproductive changes or the fate of natural preadaptations in cultivated legumes is inevitably connected with the human factor and the advent of agriculture. It is probable that pulses already had been collected and occasionally raised by prehistoric gatherer-hunters as deduced by Harris (1984). We define domestication not merely as an introduction of a wild type into cultivation but as the whole process by which a cultivated entity, adjusted to human needs and to the farm ecosystem, is formed. A perspective on this process can best be gained from archeological data. In Table 2, based on many available sources (Evans, 1968; Helbaek, 1959, 1966, 1969, 1970; Hopf, 1969, 1983; van Zeist, 1970, 1972, 1981; Hillman, 1972, 1975; Stewart, 1976; Hansen & Renfrew, 1978; Kroll, 1979; Halstead & Jones, 1980; Körber-Grohne, 1981; Kislev, 1985; Rollefson, 1985; van Zeist & Bakker-Heeres, 1985), we have tried to summarize the findings of seeds in archaeological sites in the Near East (Greece to Iran) back to 8000 B.P. There are some reservations regarding the significance of these findings. One concern is the reliability of identification. Charred seeds usually have lost their seed coats with their diagnostic texture. Even when the seed coat pattern is somehow preserved, it is difficult to distinguish between the domesticated and the wild types. Another concern is partial information—reports without any quantification.

The best criterion left for distinguishing between a domesticate and its close wild relatives is seed quantity. Large quantities, like those reported by Hopf (1969, 1983), van Zeist (1972), Stewart (1976), Kroll (1979), Halstead & Jones (1980), and Kislev (1985), may indicate a full introduction into regular cultivation. Using this criterion, the earliest reliable record of a domesticated pulse is dated considerably later than the first find. The same methodology could be applied to American species as well. For the same purpose one can compare the amount of variability in the archeological findings, assuming that variability is higher in collections from the wild and in primitive forms (Bennett, 1970; Dennell, 1974). For dating later introductions, old manuscripts (e.g., Egyptian or Chinese) could be helpful, assuming that the process had started a few centuries earlier (e.g., *Glycine* in Hymowitz & Newell, 1981). In total, five phases can be discerned in the history of a common domesticate, each stage but the last one covering about 3,000 years:

1. First introductions—growing of types selected from the wild for tasteful and plentiful food supply (Table 2; Kislev & Bar-Yosef, 1987);
2. Insurance of harvest—selection for fully or partly unopened pods and probably for soft-seededness (Table 2);
3. Acclimatization—selection of agroecotypes during the distribution of the crop from its center (e.g., *Pisum* and *Lens* in Körber-Grohne, 1981) or replacement of local cultivars (Hubbard, 1980; Ladizinsky et al., 1983);
4. Productivity—further selection for better cultivars with larger seeds and pods;
5. Standardization (homogeneity of crops, breeding for large-scale agriculture) and specialization (selection for special adaptations; Renfrew, 1973). This phase pertains to modern agriculture. Contemporary agrotechniques may condense all phases into a short time. These phases mark the developmental history of a grain crop but should not be rigidly defined.

The first two stages are of particular interest because they directly apply to modifications of the

TABLE 2. Earliest archeological records of eastern Mediterranean pulses. (The numbers are of seeds found including those of related species; + denotes no count. For detailed literature see text.)

Site	Date (BC) (C14)	*Cicer*	*Lathyrus*	*Lens*	*Pisum*	*Vicia ervilia*	*Vicia faba*
Franchthi (Paleolithic)	11000–7000	—	—	+	—	—	—
Abu Hureyra (Mesolithic)	9500–8500	—	—	+	—	+	—
Moureibit	8050–7550	—	—	20	—	5	—
Jericho (PPNA)	8300–7300	—	—	2	—	—	—
Aswad I	7800–7300	—	—	6	39	1	—
Çayönü (1)	7500–7200	1	—	—	45	67	—
Abu Hureyra (Neolithic)	7500–6500	12	—	+	—	—	1
Jericho (PPNB)	7300–6500	2	—	640	344	—	30
Çayönü (2–5)	7200–6500	3	1	162	114	141	—
Beidha	7000–6600	—	—	53	+	—	—
Hacilar (Aceram.)	6750	—	—	3	—	—	—
Aswad II	7000–6500	—	—	21	36	—	—
Ghoraifè I	6800–6300	3	1	3	19	1	—
Franchthi (Mesolithic)	7300–6000	—	—	+	+	—	—
Yiftahel	6500	—	—	7.4kg	—	—	2750
Alikosh (AKphase)	6450–6150	—	—	1	—	—	—
Ghoraifè II	6400–6100	—	—	—	7	—	—
Jarmo	6500–6000	—	+	+	+	—	—
Can Hasan III	6500–6000	—	—	15	—	37	—
Ain Ghazal	6200	+	—	+	+	—	—
Ramad I	6350–6050	—	—	75	25	2	—
Knossos	6100	—	—	210	—	—	—
Cape Andreas	6000	—	—	147	4	—	1
Prodromus	6000	—	200	13	281	—	—
Ramad II	6100–5800	20	—	558	143	—	—

reproductive traits during the initial steps of domestication; they also refer to the beginning of agriculture.

The background—whether ecological or social—of the "agricultural revolution," the possible causes of its advent, and its impact on civilization, have been dealt with by many authors (e.g., Ucko & Dimbleby, 1969; Wright, 1971; Flannery, 1973; Harlan et al., 1976; Wright, 1976; Bender, 1977; Cohen, 1977; Megaw, 1977; Reed, 1977; Whyte, 1977; Stemler, 1980; Ammerman & Cavalli-Sforza, 1984; Moore, 1985; Bar-Yosef, 1986). At the time of the agricultural revolution, the agronomical methods were "primitive," some of which, like sowing, were applied from past experience (Allen, 1977). Harvesting of pulses, for instance, was done by plucking (*Pisum*) or uprooting (*Lens*), the same methods that the gatherers had used (Bohrer, 1972). These speculative aspects are still being explored today.

Later on, in accordance with the eco-demographical and cultural developments, societies turned to vegetarianism (Sillen, 1981). Humans have learned to appreciate the nutritive value of the food not just its taste, and ventured to achieve a balanced diet. Surprisingly, in legumes the choice of taste has been (unconsciously) the choice of proteins. This trend of selecting more and better food characterizes the process of domestication from its third phase up until now. As will be shown, pulses have been the most suitable (or the best candidates) for introduction and selection throughout these phases.

III. Ecology of the Wild Relatives

We assume that some of the ecological features of the wild populations of species that are closely related to domesticated legumes could serve as prerequisites for their successful growing in the farm

ecosystem (Kupzow, 1980). We also assume that the climatic conditions and vegetation in the regions of domestication have changed slightly during the last 10,000 years (Ucko & Dimbleby, 1969; Butzer, 1971; Wright, 1976; Tchernov, 1980; van Zeist & Bottema, 1982; Baruch, 1983).

The ancestors of most domesticated legumes or the close relatives of others (such as *Vicia faba,* and part of *Phaseolus vulgaris;* see Table 1) are known today, as well as their geographical distribution and habitats. They grow in open woods or clearings (e.g., species of *Pisum, Phaseolus*) or in sparse subshrub or herbaceous formations (e.g., *Lathyrus, Lens, Lupinus*). These habitats are often disturbed, primarily owing to past and present human interference. The ecological amplitude of these relatives is usually wide, with populations of the more widespread species (e.g., *Lathyrus cicera, Vicia narbonensis*) found at diverse elevations, on a variety of soils, and with various water regimes.

The floristic composition in the natural communities of the relatives of pulses is typically rich in legumes varying in their degree of cover; according to records supplied by Danin (pers. comm.), *Vicia, Pisum,* and *Lens* grow in Israel in plant associations with 15% or more leguminous species, most of them annuals. For various reasons, for example mimicry, legumes usually attract legumes and many species may coexist. Likewise, leguminous weeds flourish among leguminous crops, as they probably did in ancient times (Barulina, 1930; Rowlands, 1959; Kolev, 1977; Hubbard, 1980). Similar habitats, rich in annual leguminous species, have been encountered by us in southern Turkey (e.g., open oak forests in southern East Anatolia) and in Iran (Kurdistan). In some cases (e.g., lupines in Israel), legumes have been cyclically replaced by grasses: During a few years the grass populations may expand at the expense of the decreasing legume populations, or vice versa. Many of the legumes in question grow in clusters (e.g., *Lens*) or in dense colonies (e.g., *Vicia galilaea, Lupinus* spp., *Phaseolus* spp.). This distribution pattern is advantageous for agricultural purposes where crop populations are necessarily dense.

Important ecogenetic features characteristic of several wild species include their genetic polymorphism, immense quantitative variability, and pronounced phenotypic plasticity (see Bennett, 1970). Species of *Vicia, Cicer, Glycine, Lens, Pisum,* and *Phaseolus,* show a wide range of variability, as well as phenotypic plasticity in almost all characters measured. Examples of this variation can be found in Barulina (1930), Plitmann (1967), van der Maesen (1972, 1984, 1986), Schäfer (1973), Waines (1975), Ladizinsky (1979a), Evans (1980), Hymowitz & Newell (1980), Cubero (1981), Lush & Evans (1981), Hawtin & Webb (1982), and Singh & Rachie (1985). Due to the prevalent breeding system (selfing, and see below) the genetic variability is often greater between subpopulations or populations than within.

IV. Phenology and Life Cycle

Legume crops, as well as their wild relatives, usually have a short life cycle: eight to sixteen weeks after germination their fruits and seeds reach maturity. This may be regarded as an adaptation of the progenitors to their natural habitats or a feature typical of their life form; in the wild, many of them are climbers having a relatively faster vegetative growth. In some wild species (e.g., *Lens orientalis, Vicia ervilia*) the entire cycle takes about six to eight weeks; in others, in which either the vegetative phase (e.g., *Lathyrus cicera, Pisum elatius*) or the duration of pod ripening (e.g., *Lupinus* spp.) is longer, the cycle may stretch to more than three months. Naturally, environmental conditions such as temperature and moisture may affect the longevity of each phase or the duration of the whole life cycle. Within the wild populations, most plants reach a certain developmental phase almost simultaneously; thus they simulate the situation under cultivation in which crop plants reach the various phenological stages simultaneously.

In nature, however, there is some variability because there are always a few genotypes that are either early or late in development and were therefore probably used by farmers for breeding early or late cultivars. As a result of such selection, the flowering season of some cultigens does not

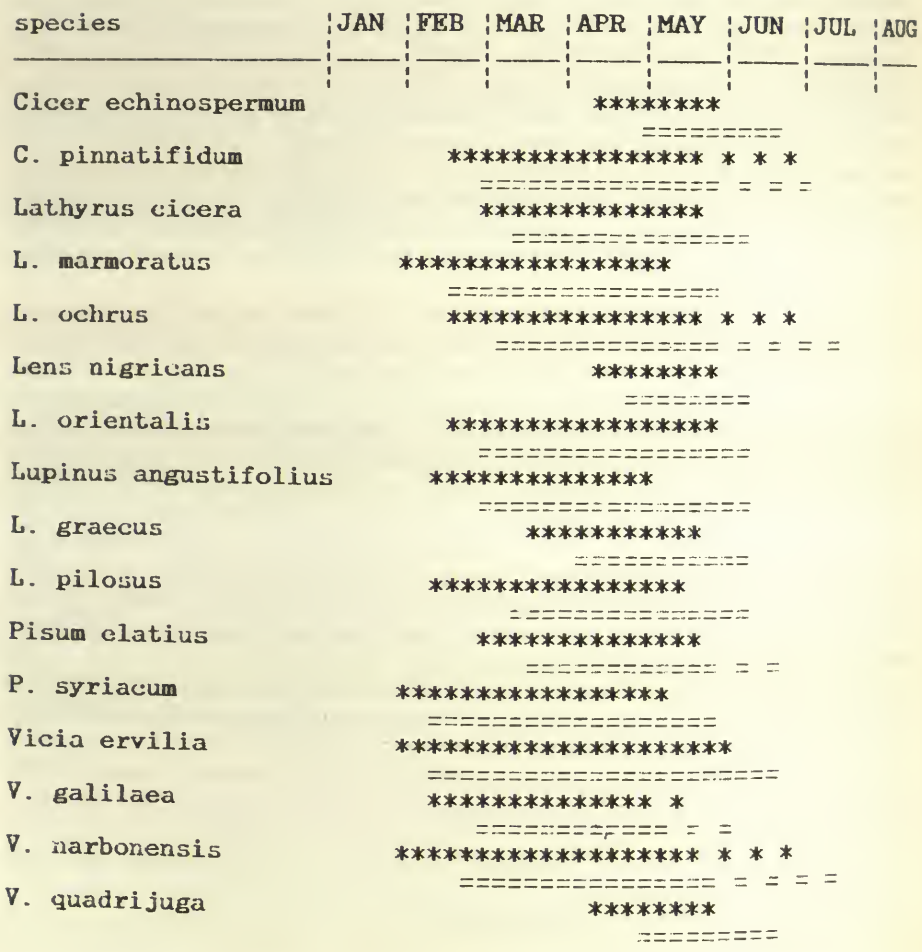

FIGURE 1. Flowering (****) and fruiting (= = =) times of the wild relatives of Old World pulses.

necessarily match that of their progenitors. Cultivated species that show considerable diversion from the "natural norm," due to artificial selection of ecotypes, include *Cicer arietinum* (fruiting times: from April–May to September) and *Lupinus albus* (April–May to July). On the other hand, species like *Lens culinaris, Vicia faba,* and *Lathyrus sativus* are still treated in the Middle East as winter crops. Cultigens or agroecotypes grown outside their original area in countries in different climatic regions (e.g., *Lathyrus sativus* in India, lupines in Australia) may have different phenological seasons. We believe that changes in the life cycle did not take place at the beginning of agriculture, and the natural phenology probably was advantageous to the people in that era.

When do the pulses' wild relatives reach their flowering and fruiting seasons? In Figure 1 we have tried to date the timing of these reproductive stages in 16 native species related to cultivated ones. Our data are based on observations, herbarium material, and relevant literature. It is evident that more than half of these species flower at the end of winter or the beginning of spring (February–March in Israel and Syria, March–April in Turkey). Species like *Lathyrus cicera, L. marmoratus, L. ochrus, Lens orientalis, Pisum syriacum, Vicia ervilia, V. galilaea, V. narbonensis,* and lupines

all bear green or ripe fruits in early spring. Even the three species that seem to start flowering later than the rest are actually growing in Anatolia or Mediterranean Europe where springtime is about a month later than in the Mediterranean Near East. Despite exhuberant growth, this part of the year offers little food. Grains of *Hordeum spontaneum* (Gramineae) reach maturity a month later, and in the case of *Triticum dicoccoides,* only in the summer (May). The first farmers, like the gatherers before them, used leguminous plants to supply fresh staple food just after winter.

One can assume that the wild progenitors of grain legumes in other regions also set fruit earlier than other exploitable food plants in months of scanty edible resources. It seems that harvest time had affected not only the process of domestication (Lynch, 1973), but also determined the first choice for that process. This could also be one of the reasons why green vegetables, sprouting at the start of spring, were later taken into cultivation in the Mediterranean region and the Middle East.

V. Breeding System

Most if not all of the domesticated pulses are self-compatible, obligate or dominant selfers (Fryxell, 1957; Free, 1970; Simmonds, 1976; Duke, 1981). Occasional cross-pollination has been observed in a few species *(Cicer arietinum, Lens culinaris, Phaseolus lunatus).* In a few others *(Lupinus albus, Phaseolus coccineus, Vicia faba)* visits of insects increase the yield of seeds, probably because tripping is needed for pollen tube penetration. A similar phenomenon was reported in wild species of *Lupinus* (Pazy, 1984). Though protandry is a common feature of both wild and domesticated legumes, pollination and fertilization can occur in the still-closed, and often very small, flower ("bud pollination"). This phenomenon was noticed in *Pisum sativum* and *Lathyrus sativus.* Since the pollen reach maturity in the papilionaceous flower at a young stage of its development, bud pollination may occur in many other cultivars. We have observed it in several wild species of the tribe Vicieae.

The advantages and consequences of selfing in cultivated crops have been discussed briefly by Fyfe (1966), Purseglove (1968), Smartt (1976b), Zohary (1984), and others for particular cases. In our view, there are three major issues of selfing that are beneficial to the breeder: (i) independence of biotic vectors in pollination, auto-production of seed; (ii) rapid selection and retention of certain genotypes; and (iii) morphological and phenological homogeneity. In all, selfing is an "insurance policy" for the quantity and quality of the product.

What are the breeding systems of the wild relatives of pulses? Much like their derived cultivars, the wild annual ancestors or relatives are self-compatible with prevalent selfing. This mode of breeding system fits their habitats, in which many of them are r-strategists. According to our observations, *Vicia ervilia, V. galilaea, V. narbonensis, V. sativa, Lathyrus marmoratus, L. pseudocicera, L. cicera, Lupinus pilosus* (to some extent), *L. angustifolius,* and local species of *Cicer, Lens,* and *Pisum* set fruit in the greenhouse after self-pollination. In nature, occasional crossing may occur, causing a "recombination explosion" and thus increasing variation within the population. This mode, characteristic of many wild legumes, is as effective in storing or releasing variability and preadaptive to domestication as the open recombination system which characterizes the close relatives of other crops (Gornall, 1983). From this aspect of reproduction, no change has been induced during the process of pulse domestication.

To summarize this section, the domestication of pulses did not require or dictate any significant change in phenology, flower morphology (except for possible slight changes in corolla color), pollination mode, or breeding system. The natural reproductive traits of the flowers of the ancestral stocks were already advantageous to the first farmers, and there had been no reason to alter them consciously.

VI. Seed and Pod Characters

In pulses, the seeds and/or the pods are the principally usable parts. Because of this, they were the main and direct targets of selection and breeding. There were two primary directions of selection

in pulses: to have more and better food, and to improve the agrotechnical and food processing methods. The first category includes higher production of flowers, fruits and seeds, larger seeds, possible use of green and dry material (multipurpose product), and nontoxic or sweet seeds. The second category includes efficient harvesting or gathering, preservation of viable seeds for a long time, high rate of simultaneous germination, and easy sowing.

As pointed out before, it is impossible to evaluate quantitatively the physical changes in seeds from the Neolithic or before, owing to the nature of the archeological material. Hypotheses may be made from comparisons of wild and primitive forms with their relatives or by deductions from later trends of breeding in primitive agriculture.

Particular changes in size or number, and sometimes chemical contents of seeds, have been described for most genera of common pulses: *Phaseolus* (Smartt, 1969, 1976a; Evans, 1980; Kaplan, 1981), *Cicer* (van der Maesen, 1972), *Lens* (Ladizinsky, 1979a), *Lupinus* (Gladstones, 1974; Baer & Gross, 1977; Hondelmann, 1984), *Pisum* (Waines, 1975), *Vicia faba* (Cubero & Suso, 1981), and *Vigna* (Lush & Evans, 1981). A few of these studies deal also with the changes in dimensions of the pod and its shape, and the anatomy of the valves and their performance. In general, the changes caused by domestication were morphological and anatomical, physiological and chemical, functional and "behavioral." Since pod and seed represent a whole adaptive strategy (see Harper et al., 1970), modification of one character could cause a chain of modifications when each change is accompanied by or associated with other changes. For example, increase in seed weight may affect dispersal, seedling establishment, and development (Stebbins, 1976); reduction of toxins associated with decrease of seed size and increase in number, or the opposite trend, may affect the rate of predation by various animals (Janzen, 1969).

Perhaps the most obvious syndrome of changes is related to the decrease in seed coat thickness. Anatomically, such a decrease is caused by a reduction in the palisade layer (Barulina, 1930; Red'kina, 1976; Patel, 1976; Werker et al., 1979; Watad, 1980). Examination of seed coat texture in various wild types and cultivars (see Heyn & Herrnstadt, 1977; Saint-Martin, 1978; Newell & Hymowitz, 1978; Miege & Mascherpa, 1979; Lush & Evans, 1980; Lersten & Gunn, 1981; Kislev & Plitmann, in prep.) reveals a trend toward a smoother texture of testa in soft seeds, with an obliteration—in different degrees—of the SEM pattern. In *Cicer* this change is macroscopic; in *Lathyrus* it is scarcely obvious. In addition, many of the cultivars have seed coats with lighter colors (white, yellowish, and greenish). This change is linked with the reduction of phenolics in the testa (Werker et al., 1979).

These physical and chemical changes affect the physiological behavior and the biological functioning of the seed in the following ways: faster imbibition, which increases the rate of germination or shortens the time of cooking; less protection (e.g., against bruchids or fungal attack); and better taste. Furthermore, by soaking the seeds and then cooking them, humans could remove toxic substances (Leopold & Ardrey, 1972), such as alkaloids, from lupines. Apparently, storage bruchids did not cause much damage to stored seeds in the ancient Middle East and Europe (Kislev, unpublished) and therefore the unprotected seeds could be stored and preserved for a long time. Another significant change was the increase in seed size and seed weight. Large and good-tasting seeds were the first choice in selection for edible pulses. The Vicieae and the Phaseoleae contain herbaceous species that have large seeds. Large-seeded annuals establish themselves on poor soils and in herbaceous formations better than small-seeded annuals. However, artificial selection for even larger seeds has continued throughout the process of domestication, especially in phase 4. As a result, seeds of domesticates are much heavier than those of their wild counterparts. As expected, the parameters of size and weight are positively correlated (Table 3). Following a study on defense mechanisms in wild legume populations (Watad, 1980), we have found that seed weight or size shows a significant positive correlation with thickness of testa; it is evident separately in wild and cultivated types (Table 3; Fig. 2). On the other hand, by clustering analysis we find out that 78% of the cultivated strains have seed coats thinner than 0.15 mm, and 70% of the wild populations have seed coats thicker than 0.16 mm. Generally, the increase in size was not accompanied by a

TABLE 3. Correlations between various physical characters of the seed.

Character pair	Number of samples	Correlation coefficient	Slope	Intercept
Seed length ×				
Seed weight	40	0.9252*	0.0832	−0.3855
Seed coat thickness ×				
Seed length				
domesticates	20	0.7530*	71.6213	0.4883
wild types	20	0.6863*	10.5066	2.7864
Seed coat thickness ×				
Seed weight (Fig. 2)				
domesticates	24	0.7126*	6.8280	−0.4992
wild types	22	0.7932*	0.3641	−0.0017

*Significant at $P = 0.01$

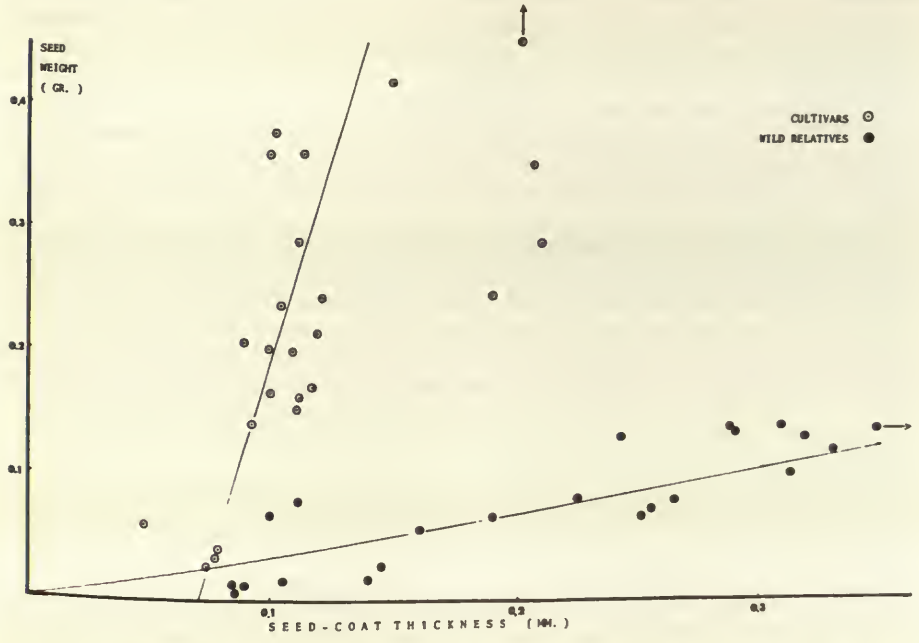

FIGURE 2. Seed weight as correlated with seed coat thickness in legume cultivars and their related wild species. Cultivars (circled dots) include: *Cicer arietinum* (2 cultivars), *Glycine max* (1), *Lathyrus sativus* (3), *Lens culinaris* (3), *Lupinus albus* (2), *L. mutabilis* (2), *Pisum sativum* (3), *Phaseolus vulgaris* (4), *Vicia ervilia* (1), *V. faba* (2+1). Wild relatives (solid dots) include: *Cicer* (3 species), *Lathyrus* (2), *Lens* (2), *Lupinus* (2), *Pisum* (3), *Vicia* (3 species).

change in shape. Excluding perhaps *Vicia faba*, seeds of domesticates resemble those of their relatives. Moreover, positive significant correlations between seed length and width were found within 21 out of 26 samples or populations of the studied material. Similar results were obtained by Katayama (1979) and others. This means that seed dimensions co-vary with one another. A significant change in shape is expected to result from mutation (as known in *Pisum* or *Lathyrus*) independently of an induced change in size. Other correlations involving seed weight, number of

TABLE 4. Indices of relative change in seed characters of domesticated pulses and their relatives. (See text for an explanation of domestication index.)

Domesticate	Ancestor/related species	Domestication index
Cicer arietinum	*C. reticulatum*	9.9–10.0
C. arietinum	*C. echinospermum*	10.7–11.0
Lathyrus sativus	*L. cicera*	3.3–3.4
L. sativus	*L. marmoratus*	6.9–7.0
Lens culinaris	*L. orientalis*	14.7–15.0
(large-seeded)	*L. nigricans*	12.2–12.3
L. culinaris	*L. nigricans*	3.3–3.4
(small-seeded)	*L. orientalis*	4.0–4.1
Lupinus albus	*L. graecus*	2.4–2.5
L. mutabilis (d)	*L. mutabilis* (wild)	5.3–5.4
Pisum sativum	*P. syriacum*	6.3–6.4
P. sativum	*P. elatius*	6.0–6.1
P. sativum	*P. fulvum*	6.5–6.6
Vicia ervilia (d)	*V. ervilia* (wild)	3.0–3.1
V. faba (large)	*V. galilaea*	17.5–17.6
V. faba (large)	*V. narbonensis*	18.9–19.0
(small-seeded)	*V. narbonensis*	3.4–3.5

cells in the cotyledones, mean cell mass, and DNA amount appear in the genus *Vicia* (Davies, 1977).

An increase in seed size is associated with an increase in pod size. Neither change affected the number of seeds per pod, which remained more or less constant: domesticates have about the same number as their wild relatives. Since we are dealing with selfers, their breeding system, which is considered to be efficient, ensures the fertilization of most ovules. Certain cultivars of *Pisum sativum*, however, have a number of seeds per pod greater than the average in nature (9 vs. 6), but the natural variability in the progenitors (4–9 seeds per pod) afforded this selection. In *Vicia faba* there is some reduction in number (average of 3–4 per pod) compared with its wild relatives (4–6 seeds per pod), but because the direct ancestor of this species is still unknown, we cannot attribute this reduction only to a trade-off mechanism.

Testa thickness has been reduced by 10–25% in most species, by 45–50% in *Lens culinaris*, and by over 50% only in *Pisum sativum* and *Cicer arietinum*. These rates are unrelated to the initial thickness. In the large-seeded cultivars of *Vicia faba* and *Lens culinaris*, the net gain in seed weight (the weight of the cotyledons) was over 500%, in *Lupinus mutabilis* 300–500%, and in the rest 200–300% (excluding *Vicia ervilia* and *Lupinus albus* with an increase of 100–200%). Both changes are equally significant. Thus, we can find an index that combines, by simple multiplication, the ratio of change in seed weight with the ratio of change in seed coat thickness:

$$\text{"domestication index"} = \frac{\text{seed weight (domestic)}}{\text{seed weight (wild)}} \times \frac{\text{coat thickness (wild)}}{\text{coat thickness (domestic)}}$$

The domestication index provides a simplistic way to gain some idea of the relative rate of change in certain characters that are important in cultivation. We have calculated the indices for Middle Eastern pulses (Table 4). *Vicia faba*, *Lens culinaris*, and *Cicer arietinum* manifest the greatest change; *Lupinus albus* and *Vicia ervilia* display the lowest. That also could be the distinction between primary and secondary crops. When applied to pairs of related wild species, the indices are only 1.0 to 2.1. The indices proposed here represent the relative change in seed characters during domestication; one can apply, of course, other formulas to convey the same idea.

VII. Dispersal and Defense

All the ancestral stocks of today's pulses are autochorous (Fahn & Zohary, 1955; van der Pijl, 1972). The main change in pod, induced during the first stages of domestication, was anatomical–functional, i.e., selection of partly or fully nondehiscing pods (Smartt, 1976b; Karpitskaya & Taranukho, 1976; Müntz et al., 1978; Ladizinsky, 1979b). We assume the goal was not necessarily to obtain a complete nonshattering of seeds but rather a delayed dehiscence of the fruits. Such pods could be readily collected and eventually threshed or, in time, the seeds could be easily picked or collected, and stored. Considering the natural variability, this goal was easier to achieve. Moreover, in many pods with an effective twisting mechanism of valves, a few seeds (often the basal ones) remain in the opened pod (Plitmann, 1986). In addition, pods of some pulses are eaten when green and fresh, just before ripening (e.g., *Pisum sativum*). As in many other legumes, the fully developed seeds can reach maturity in the picked pod and be used later for several purposes.

According to Watad (1980), the common defense mechanisms in diaspores of annual legumes in the eastern Mediterranean region are either morphoanatomical characters of the seed coat, where seeds are the propagules, or traits of the valves in unopened pods. In the latter case the seeds are comparatively small and have a thin testa. The breakability of the coat, measured as the "threshold of cracking," is positively correlated with its thickness. Watad supposed that secondary substances play a minor role in this respect. He noted that while small seeds (usually of indehiscent pods) do not have harmful substances such as alkaloids or cyanogenic compounds, larger seeds may contain those or others, e.g., nonprotein amino acids in species of *Lathyrus* and *Vicia*, in just enough quantity to avoid consumption by small predators. Similar relations are found in the cultivated pulses.

The chemical changes in the seeds are complex. Many pulses, like their progenitors, are practically nontoxic. Even if they contain bitter or toxic compounds these are in small quantities and harmless to large mammals. In a few species, such as lupines and *Lathyrus sativus*, the presence of such compounds did present some handicap. In these species, nontoxic or sweet cultivars could have been obtained only by time-consuming modern breeding methods. Therefore, such changes belong to the fifth phase of domestication. Other chemical changes have been achieved through breeding of strains with fat-rich seeds or with better protein quality. This selection, though probably easier and faster than the above, still marks a modern trend (e.g., in Delane, 1987).

A delayed shattering or nondehiscence adds some partial protection to the seeds. This advantage has become even more important in the subsequent phases of domestication when selection for thinner testa and/or nontoxic seeds takes place. Extra protective measures have been lent to the embryo by human techniques of preservation and maintenance.

To conclude this section, the main changes induced by domestication in seed and pod are expressed in their physical properties which affect in various degrees dispersal, defense, germination, and establishment.

VIII. Why the Legumes?

Several papers and books describe and discuss general models of the origins of agriculture and the process of plant domestication (e.g., Ucko & Dimbleby, 1969; Wright, 1971; Flannery, 1973; Lynch, 1973; Cohen, 1977; Lawton & Wilke, 1977; Reed, 1977; Hubbard, 1980; Barigozzi, 1986). In Kupzow (1980) two modes are defined: naturalization and acclimatization. The domestication of pulses involves both modes, successively or simultaneously. During the first two phases of this process the pulses were "naturalized." Because the farm presents a set of ecological conditions different from nature and with effective biotic factors, we may regard it as a new environment. Therefore, the legumes introduced into it were artificially adapted and genetically changed. Acclimatization of legumes has been accomplished during the third and fifth phases of domestication.

Still, owing to the "weedy" habit of many, some legumes could grow in secondary habitats in new regions without considerable change. Moreover, as indicated by Smartt (1976a, b) for *Phaseolus*, and Plitmann (1967) for *Vicia* (following Vavilov's principle of homologous series), legumes demonstrate parallel homology. They may occupy similar ecological niches in different climatic zones and respond to selective pressures in convergent adaptations. The ecological adaptations and demographical attributes of the ancestral stocks are part of a set of preadaptations that gave the grain legumes their predominance in cultivation.

The significant preadaptations in the initial phases of domestication are: time of pod setting; self-compatibility and prevalent selfing; easy harvesting or collecting of large quantities of seeds, for immediate use as well as for storage for food and sowing; taste and size of seeds, and sometimes pods. Large-seeded annual species with such valuable qualities are found in the Vicieae and the Phaseoleae, and to some extent in *Cicer* and *Lupinus*.

Consequently, we may conclude that the domestication of pulses was characterized by reproductive "unchanges" rather than by changes. Moreover, most of these characters have not been altered or diverged considerably since the first introductions, though some modifications (e.g., in phenology or pollination mode) have been established later by breeding procedures. Changes induced by the domestication of pulses, though few, are biologically significant: indehiscence of pod in various degrees; reduction in seed coat thickness, with all the physiological implications; and additional increase in seed size and weight, and pod size (valves), culminating in a "monstrous" seed like that of *Vicia faba*. The wide variability and relatively simple and rapid selection for those characters were advantageous for the farmer and the breeder. Breeding experiments in lupines in South America, Australia, and South Africa (C. Stirton, pers. comm.), are recent examples of the induction of large changes in seed characters in a relatively short time. Both reproductive changes and unchanges in pulses were probably conscious (as termed by Pickersgill & Heiser, 1976).

The artificial selection in pulses was primarily for seed predation by humans, as were the changes induced in seed and pod characters. Moreover, whereas other modifications may be compatible with natural trends, the changes in pod and seed which impinge upon biological functioning did counter natural trends. The plant lost its ability to disperse and the progeny lost its defense. These two functions have been taken over by humans. This combination of many preadaptations in characters prerequisite for cultivation and readily modified seed/pod characters (within a few generations of artificial selection) may be the answer to the question, "Why the legumes?"

Among the pulses, one can find different levels of usage, adoption, or abandonment. Species with high indices have been—and still are—primary crops (broad beans, lentils, chick-peas, and peas; FAO Production Yearbook, 1982). Species with indices of domestication lower than 4.0 are either secondary crops (e.g., *Lathyrus sativus, Lupinus albus*) or have been replaced and neglected (*Vicia ervilia, Lens nigricans*, and *Lathyrus cicera*). Other legumes that could have been exploited at one time or another, particularly in time of starvation, show an incomplete set of characters. Such legumes include *Lathyrus ochrus, Lupinus pilosus, Vicia michauxii*, and *Vicia peregrina*, which have comparatively large seeds but also pods that ripen late and/or dehisce, and seeds that are either untasteful, toxic, or furnished with a thick testa. Changes in these species were probably more difficult to induce. Other potential candidates which could be used for food in springtime are found in the Compositae (*Atractylis, Cynara*), the Malvaceae (*Malva*), the Liliaceae (*Allium*), and perhaps some Cruciferae. None of these, however, have the combination of qualities of the grain legumes.

In this respect, it is interesting to compare the advantages and disadvantages of pulses over grain cereals. The common shifts in cereals have been in life form (perennials to annuals, e.g., rice and possibly rye), in breeding system (crossers to selfers, e.g., rice, rye, sorghum; dioecious to monoecious: corn), in dispersal mode (shattering to nonshattering), and reproductivity (barley, corn, oats, wheat). The grains seldom contain toxic or inhibition compounds. Moreover, grasses are used for many other purposes than food. The changes in legumes were seemingly fewer or smaller, probably owing to their preadaptive combination. Their early fruiting was important to the first farmers, who, besides, were able to grow them on poor soils and thus enrich the soil. Other

characters such as habit, susceptibility to pests, and some bitterness of seeds, were of secondary importance. These differences can be attributed to an older and longer period of domestication of cereals—a hypothesis that is contradicted by the accumulating archeological and palaeoethnobotanical evidence. At any rate, this same process in pulses has proceeded long enough to induce certain changes when needed. Hence, the alternative interpretation is more plausible: pulses and cereals have been under dissimilar forces and courses of artificial selection, complying with the different genetic and reproductive background of the exploited plants. Similarly, the methods of selection and breeding could have been different as well. Natural hybridization is more common among grasses, partly due to open wind pollination, than in cultivated legumes or their wild relatives (Smartt, 1979, 1980). Thus the changes in pulses are usually the outcome of a simple choice of certain phenotypes. In cereals, this process involved selection of hybrids and gross chromosomal changes. Discussions on the evolution of grain crops are usually based on the situation in grasses (Harlan, 1970; Zohary, 1984). Differentiation–hybridization cycles or stabilizing selection are not typical of the whole domestication process of pulses. It seems that for most pulses, the process started as directional selection (phases I and II), proceeded as stabilizing selection (phases III, IV) and presently may be regarded as diversifying selection.

In this paper we have not discussed recent reproductive changes induced by domestication. These changes are numerous and specific, in accordance with the diverse aims of breeding (see Sneep & Hendriksen, 1979). New methods are applied or suggested, particularly in order to overcome the genetic isolation of grain legumes (Razdan & Cocking, 1981). Old domesticates are improved and new introductions are tried (USA Natl. Acad. Sci., 1979; Hawtin & Chancellor, 1979; Thompson, 1981; Hedley et al., 1983; McIvor & Bray, 1983; Thompson & Casey, 1983; Whitcombe & Erskine, 1984; Hossaert et al., 1985; Singh & Rachie, 1985; Wickens et al., 1985). These trials may result in cultivars that have been genetically changed in physiology and ecology; in vegetative architecture; in reproductive traits such as flower size and fertility; in productivity; in chemistry of seeds; and in resistance to pests.

For all these developments, the wild genetic resources of legumes are important because they are the major donors of germplasm for further changes adaptive to human ecology. The processes of domestication of pulses, their retained unchanges, and ensuing changes, may serve as a model for future trials.

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References

Adams, M. W. & J. J. Pipoly. 1980. Biological structure, classification and distribution of economic legumes. Pp. 1–16 *in* R. J. Summerfield & A. H. Bunting (editors), Advances in Legume Science. Royal Botanic Gardens, Kew.

Allen, O. N. & E. K. Allen. 1981. The Leguminosae. Univ. of Wisconsin Press, Madison, Wisconsin.

Allen, T. F. H. 1977. Neolithic urban primacy: the case against the invention of agriculture. J. Theor. Biol. 66: 169–180.

Ammerman, A. J. & L. L. Cavalli-Sforza. 1984. The Neolithic Transition and the Genetics of Populations in Europe. Princeton Univ. Press, Princeton, New Jersey.

Baer, E. von & R. Gross. 1977. Auslese bitterstoffamer Formen von *Lupinus mutabilis*. Zt. Pflanzenzücht 79: 52–58.

Baker, H. G. 1972. Human influences on plant evolution. Econ. Bot. 26: 32–43.

Barigozzi, C. (editor). 1986. The Origin and Domestication of Cultivated Plants. Elsevier, Amsterdam.

Baruch, U. 1983. The palynology of a late holocene core from lake Kinneret. MS thesis. The Hebrew University, Jerusalem. [In Hebrew.]

Barulina, H. 1930. Lentils of the USSR and of other countries. Bull. Appl. Bot. Genet. & Pl. Breed. Suppl. 40: 265–319.

Bar-Yosef, O. 1986. The origin of agricultural communities in the Jordan Valley. Rotem 20: 150–157. [In Hebrew.]

Bender, B. 1977. Farming in Prehistory: From Hunter-gatherer to Food Producer. J. Baker, London.

Bennett, E. 1970. Adaptation in wild and cultivated plant populations. Pp. 115–129 *in* O. H. Frankel & E. Bennett (editors), Genetic Resources in Plants. Blackwell, Oxford.

Bohrer, V. L. 1972. On the relations of harvest methods to early agriculture in the Near East. Econ. Bot. 26: 145–155.

Butzer, K. W. 1971. Environment and Archeology. Methuen, London.

Cohen, M. N. 1977. The Food Crisis in Prehistory. Yale Univ. Press, New Haven, Connecticut.

Cubero, J. I. 1981. Origin, taxonomy and domestication. Pp. 15–38 *in* C. Webb & B. Hawtin (editors), Lentils. Commonw. Agric. Bur., Slough, U.K.

_____ & M. J. Suso. 1981. Primitive and modern forms of *Vicia faba*. Kulturpflanze 29: 137–145.

Davies, D. R. 1977. DNA contents and cell number in relation to seed size in the genus *Vicia*. Heredity 39: 153–163.

Delane, R. (editor). 1987. Proceedings of the 4th International Lupin Conference. ILA, Geraldton, Australia.

Dennell, R. W. 1974. The purity of prehistoric crops. Proc. Prehist. Soc. 40: 132–135.

Duke, J. A. 1981. Handbook of Legumes of World Economic Importance. Plenum Press, New York.

Evans, A. M. 1980. Structure, variation, evolution and classification in *Phaseolus*. Pp. 337–347 *in* R.J. Summerfield & A.H. Bunting (editors), Advances in Legume Science. Royal Botanic Gardens, Kew.

Evans, J. D. 1968. Knossos Neolithic, Part II. Ann. British School Athens 63: 239–276.

Fahn, A. & M. Zohary. 1955. On the pericarpial structure of the legumen, its evolution and relation to dehiscence. Phytomorphlogy 5: 99–111.

FAO. 1982 FAO Production Yearbook. FAO, Rome.

Flannery, K. V. 1973. The origins of agriculture. Ann. Rev. Anthropology 2: 271–310.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, New York.

Fryxell, P. A. 1957. Mode of reproduction of higher plants. Bot. Rev. 23: 135–233.

Fyfe, J. L. 1966. The reproductive biology in relation to plant breeding. Pp. 40–52 *in* J.G. Hawkes, (editor), Reproductive Biology and Taxonomy of Vascular Plants. Pergamon Press, London & New York.

Gladstones, J. S. 1974. Lupins of the Mediterranean region and Africa. Dept. Agric. W. Australia, Techn. Bull. 26.

Gornall, R. J. 1983. Recombination systems and plant domestication. Biol. J. Linn. Soc. 20: 375–383.

Halstead, P. & G. Jones. 1980. Early neolithic economy in Thessaly—some evidence from excavations at Prodromos. Anthropologika 1: 93–117.

Hansen, Y. & J. M. Renfrew. 1978. Paleolithic-neolithic seed remains at Franchti cave, Greece. Nature 271: 349–352.

Harlan, J. R. 1970. Evolution of cultivated plants. Pp. 19–32 *in* O.H. Frankel & E. Bennett (editors), Genetic Resources in Plants. Blackwell, Oxford.

_____, J. M. J. De Wet & A. B. L. Stemler (editors). 1976. Origins of African Plant Domestication, Mouton, The Hague.

Harper, J. L., P. H. Lovell & K. G. Moore. 1970. The shapes and sizes of seeds. Ann. Rev. Ecol. Syst. 1: 327–356.

Harris, D. R. 1984. Ethnohistorical evidence for the exploitation of wild grasses and forbs: its scope and archeological implications. Pp. 63–69 *in* W. van Zeist & W. A. Casparie (editors), Plants and Ancient Man. Balkema, Rotterdam & Boston.

Hawtin, G. C. & G. J. Chancellor (editors). 1979. Food Legume Improvement and Development. I. D. R. C., Ottawa.

_____ & C. Webb (editors). 1982. Faba Bean Improvement. Martinus Nijhoff, The Hague.

Hedley, C. L., M. J. Ambrose & K. A. Pyke. 1983. Developing an improved plant model for the pea crop. Pp. 135–146 *in* D. G. Jones & D. R. Davies (editors), Temperate Legumes. Pitman, Boston & London.

Helbaek, H. 1959. Domestication of food plants in the Old World. Science 130: 365–372.

_____. 1966. Pre-pottery Neolithic farming at Beidha: a preliminary report. Palest. Explor. Quart. 98: 61–66.

_____. 1969. Plant collecting, dry farming and irrigation agriculture in prehistoric Deh Luran. Pp. 383–426

in F. Hole, K. V. Flannery & A. J. Neely (editors), Prehistory and Human Ecology of the Deh-Luran Plain. Ann Arbor, Michigan.

———. 1970. The plant husbandry of Hacilar: A study of cultivation and domestication. *In:* J. Mellaart (editor), Excavations at Hacilar, 1: 189–244. Edinburgh Univ. Press.

Heyn, C. C. & I. Herrnstadt. 1977. Seed coat structure of Old World *Lupinus* species. Bot. Notis. 130: 427–435.

Hillman, J. C. 1972. Plant remains. Pp. 182–188 *in* E. Higgs (editor), Papers in Economic Prehistory. Cambridge Univ. Press, Cambridge, England.

———. 1975. The plant remains from Tel Abu Hureyra: A preliminary report. Proc. Prehist. Soc. 41: 70–73.

Hondelmann, W. 1984. The lupin—ancient and modern crop plant. Theor. Appl. Genet. 68: 1–9.

Hopf, M. 1969. Plant remains and early farming in Jericho. Pp. 355–359 *in* P. J. Ucko & G. W. Dimbleby (editors), The Domestication and Exploitation of Plants and Animals. Duckworth, London.

———. 1983. Jericho plant remains. *In:* K. M. Kenyon & T. A. Holland (editors), Excavations at Jericho. 5: 576–621. British School of Archeology Jerusalem, London.

Hossaert, M., M. Delbos & D. Combes. 1985. Interet agronomique de quelques *Lathyrus*. Les gesses pour l'alimentation humaine. C. R. Acad. Agr. France 71: 65–72.

Hubbard, R. N. L. B. 1980. Development of agriculture in Europe and the Near East: evidence from quantitative studies. Econ. Bot. 34: 51–67.

Hymowitz, T. & C. A. Newell. 1980. Taxonomy, speciation, domestication, germplasm resources and variation in the genus *Glycine*. Pp. 251–264 *in* R. J. Summerfield & A. H. Bunting (editors), Advances in Legume Science. Royal Botanic Gardens, Kew.

——— & ———. 1981. Taxonomy of the genus *Glycine*, domestication and uses of soybean. Econ. Bot. 35: 272–288.

Janzen, D. H. 1969. Seed eaters versus seed size, number, toxicity and dispersal. Evolution 23: 1–27.

Jones, D. G. & D. R. Davies (editors). 1983. Temperate Legumes: Physiology, Genetics and Nodulation. Pitman, Boston & London.

Kaplan, L. 1981. What is the origin of the common bean? Econ. Bot. 35: 240–254.

Karpitskaya, N. & G. Taranukho. 1976. Anatomical structure and pod shattering in lupin. Genetika, Selektsiya i Semenovodstvo, Sbor. Nauch. Trud. Beloruss. Sel'sk. Akad. 13: 14–22. [In Russian.]

Katayama, T. C. 1979. Some morphological characters of pulses collected in the Indian sub-Continent. Mem. Fac. Kogoshima Univ. 15: 29–48.

Kislev, M. E. 1985. Early Neolithic horsebean from Yiftahel, Israel. Science 228: 319–320.

———. Archaeobotany and storage archaeoentomology. *In* J. Renfrew (editor), New Light on Ancient Farming. Univ. Press, Edinburgh (forthcoming).

——— & O. Bar-Yosef. 1988. The legumes: the earliest domesticated plants in the Old World? Curr. Anthrop. 29: 175–179.

Kolev, I. D. 1977. Mimetism in plants. Rasteniev''dni Nauki 14: 49–58. [In Bulgarian.]

Körber-Grohne, U. 1981. Pflanzliche Abrücke in eizenzeitlicher Keramik—Spiegelbild damaliger Nutzpflanzen? Fundberichte aus Baden-Württemberg 6: 165–211.

Kroll, H. 1979. Kulturpflanzen aus Dimini. Archaeo-Physika 8: 173–189.

Kupzow, A. J. 1980. Theoretical basis of the plant domestication. Theor. Appl. Genet. 57: 65–74.

Ladizinsky, G. 1979a. The origin of lentil and its wild gene pool. Euphytica 28: 172–187.

———. 1979b. Seed dispersal in relation to the domestication of Middle East legumes. Econ. Bot. 33: 284–289.

———, D. Braun & F. J. Muehlbauer. 1983. Evidence for domestication of *Lens nigricans* (M.B.) Godron in S. Europe. Bot. J. Linn. Soc. 87: 169–176.

Lawton, H. W. & P. J. Wilke. 1977. Ancient agricultural systems in dry regions. Pp. 1–44 *in* A. E. Hall, G. H. Connell & H. W. Lawton (editors), Agriculture in Semi-Arid Environments. Springer, Berlin & New York.

Leopold, A. C. & R. Ardrey. 1972. Toxic substances in plants and the food habits of early man. Science 176: 512–514.

Lersten, N. R. & C. R. Gunn. 1981. Seed morphology and testa topography in *Cicer* (Fabaceae: Faboideae). Syst. Bot. 6: 223–230.

Lush, W. M. & T. Evans. 1980. The seed coats of cowpeas and other grain legumes: structure in relation to function. Field Crops Research 3: 267–286.

——— & ———. 1981. The domestication and improvement of cowpeas (*Vigna unguiculata* (L.) Walp.). Euphytica 30: 579–587.

Lynch, T. F. 1973. Harvest time, transhumance and the process of domestication. Amer. Anthrop. 75: 1254–1259.

Maesen, L. J. G. van der. 1972. *Cicer*. A monograph on the genus with special reference to chickpea (*C. arietinum*), its ecology and cultivation. Meded. Landbouwhogesch. Wageningen 76: 1–136.

———. 1984. Taxonomy, distribution and evolution of chickpea and its wild relatives. Pp. 95–104 *in* J. R. Whitcombe & W. Erskine (editors), Genetic Resources and their Exploitation: Chickpeas, Fababeans, Lentils. Martinus Nijhoff, The Hague.

———. 1986. *Cajanus* DC. and *Atylosia* W. & A. (Leguminosae). Agric. Univ. Wageningen papers 84–85.

McIvor, J. G. & R. A. Bray (editors). 1983. Genetic Resources of Forage Plants. CSIRO, Melbourne.

Megaw, J. V. S. (editor). 1977. Hunters, Gatherers and First Farmers beyond Europe. Leicester Univ. Press, Leicester.

Miège, J. & J. M. Mascherpa. 1979. Etude des surfaces tégumentaires des graines de quelques Phaseoleae: méthodologie. Candollea 34: 87–97.

Moore, A. M. T. 1985. The development of Neolithic societies in the Near East. Advances in World Archeology No. 4.

Müntz, K., A. Rudolph, G. Schlesier & P. Silhengst. 1978. The function of the pericarp in fruits of crop legumes. Kulturpflanze 26: 37–67.

Newell, C. A. & T. Hymowitz. 1978. Seed coat variation in *Glycine* Willd. subgenus Glycine (Leguminosae) by SEM. Brittonia 30: 76–88.

Patel, J. D. 1976. Comparative seed coat anatomy of some Indian pulses. Phyton (Austria) 17: 287–299.

Pazy, B. 1984. Insect-induced self-pollination. Pl. Syst. Evol. 144: 315–320.

Pickersgill, B. & C. B. Heiser. 1976. Cytogenetics and evolutionary change under domestication. Phil. Trans. Roy. Soc. London B, 275: 55–69.

Pijl, L. van der. 1972. Principles of Dispersal in Higher Plants. Springer, Berlin & New York.

Plitmann, U. 1967. Biosystematic studies in the annual species of *Vicia* and *Lathyrus* of the Middle East. Ph.D. dissertation. The Hebrew University, Jerusalem. [In Hebrew, English summary.]

———. 1986. Alternative modes in dispersal strategies. Proc. Roy. Soc. Edinburgh 89B: 193–202.

Purseglove, J. W. 1968. Tropical Crops. Dicotyledons (1). Longmans, London.

Razdan, M. K. & E. C. Cocking. 1981. Improvement of legumes by exploring extra-specific genetic variation. Euphytica 30: 819–833.

Red'kina, Z. V. 1976. Anatomy and germination of hard seeds of some legumes. Byull. Inst. Rasteniev. N. I. Vavilova 62: 72–77. [In Russian.]

Reed, C. A. (editor). 1977. Origins of Agriculture. Mouton, The Hague.

Renfrew, J. M. 1973. Palaeoethnobotany. The Prehistoric Food Plants of the Near East and Europe. Columbia Univ. Press, New York.

Rollefson, G. O. 1985. The 1983 season at the Early Neolithic site of Ain Ghazal. Natl. Geogr. Res. 1: 44–62.

Rowlands, D. G. 1959. A case of mimicry in plants—*Vicia sativa* L. in lentil crops. Genetica 30: 435–446.

Saint-Martin, M. 1978. Observations séminologiques au microscope e'lectronique a' balayage de diverses especes de Papilionace'es. Comptes Hebdomadaires des Seances de l'Academie des Sciences, D. 287: 927–930.

Schäfer, H. I. 1973. Zur Taxonomie der *Vicia narbonensis*-Gruppe. Kulturpflanze 21: 211–273.

Sillen, A. 1981. Strontium and diet at Hayonim Cave. Amer. J. Phys. Anthropol. 56: 131–137.

Simmonds, N. W. (editor). 1976. Evolution of Crop Plants. Longman, London.

———. 1979. Principles of Crop Improvement. Longman, London.

Singh, S. R. & K. O. Rachie (editors). 1985. Cowpea Research, Production and Utilization. J. Wiley & Sons, New York.

Smartt, J. 1969. Evolution of American *Phaseolus* beans under domestication. Pp. 451–462 *in* P. J. Ucko & J. W. Dimbleby (editors), The Domestication and Exploitation of Plants and Animals. Duckworth, London.

———. 1976a. Tropical Pulses. Longman, London.

———. 1976b. Comparative evolution of pulse crops. Euphytica 25: 139–143.

———. 1978. The evolution of pulse crops. Econ. Bot. 32: 185–198.

———. 1979. Interspecific hybridization in the grain legumes—a review. Econ Bot. 33: 329–337.

———. 1980. Evolution and evolutionary problems in food legumes. Econ. Bot. 34: 219–235.

Sneep, J. & A. J. T. Hendriksen. 1979. Plant Breeding Perspectives. Center for Agricultural Publishing, Wageningen.

Stebbins, G. L. 1976. Seed and seedling ecology in annual legumes. I. A comparison of seed size and seedling development in some annual species. Oecol. Plant. 11: 321–331.

Stemler, A. B. L. 1980. Origins of plant domestication in the Sahara and the Nile Valley. Pp. 503–526 *in* M. A. J. Williams & H. Faure (editors), The Sahara and the Nile. Balkema, Rotterdam.

Stewart, R. B. 1976. Palaeoethnobotanical report—Cayönü 1972. Econ. Bot. 30: 219–225.

Summerfield, R. J. & A. H. Bunting (editors). 1980. Advances in Legume Science. Royal Botanic Gardens, Kew.

——— & E. H. Roberts (editors). 1985. Grain Legume Crops. Collins Professional & Technical Books, London.

Tchernov, E. 1981. The impact of the Postglacial on the fauna of Southwest Asia. Pp. 197–216 *in* W. Frey & H. P. Uerpmann (editors), Beiträge zur Umweltsgeschichte des Vorderen Orients. A/8 Beihefte zum Tübinger Atlas des Vorderen Orients, Wiesbaden.

Thompson, R. (editor). 1981. *Vicia faba:* Physiology and Breeding. Martinus Nijhoff, The Hague.

——— & R. Casey (editors). 1983. Perspectives for Peas and Lupins as Protein Crops. W. Junk, The Hague.

Ucko, P. J. & G. W. Dimbleby (editors). 1969. The Domestication and Exploitation of Plants and Animals. Aldine, Chicago, Illinois.

USA National Acad. Sci. 1979. Tropical Legumes: Resources for the Future. USA Natl. Acad. Sci., Washington, D.C.

Waines, J. G. 1975. The biosystematics and domestication of peas (*Pisum* L.). Bull. Torrey Bot. Club 102: 385–395.

Watad, A. E. 1980. Defense mechanisms in seeds and other diaspores of representative species of local legumes. MS thesis, The Hebrew University, Jerusalem. [In Hebrew.]

Webb, C. & G. Hawtin (editors). 1981. Lentils. Commonw. Agric. Bur., Slough, U.K.

Werker, E., I. Marbach & A. M. Mayer. 1979. Relations between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. Ann. Bot. 43: 765–771.

Whitcombe, J. R. & W. Erskine (editors). 1984. Genetic Resources and their Exploitation: Chickpeas, Fababeans, Lentils. Martinus Nijhoff, The Hague.

Whyte, R. O. 1977. The botanical neolithic revolution. Human Ecology 5: 209–222.

Wickens, G., D. Field & J. R. Goodwin (editors). 1985. Plants for Arid Lands. Allen & Unwin, London.

Wright, G. H. 1971. Origins of food production in southwestern Asia: a survey of ideas. Curr. Anthrop. 12: 447–477.

Wright, H. E. 1976. The environmental setting for plant domestication in the Near East. Science 194: 385–389.

Zeist, W. van. 1970. The Oriental Institute excavations at Mureybit, Syria: Preliminary report on the 1965 campaign, Part III. The Palaeobotany. J. Near Eastern Studies 29: 167–176.

———. 1972. Palaeobotanical results of the 1970 season at Çayönü, Turkey. Helinium 12: 3–19.

———. 1981. Plant remains from Cape Andreas—Kastros (Cyprus). Pp. 95–100 *in* A. le Brun (editor), Un Site neolithique preceramique en Chypre: Cap Andreas—Kastros. Edition A. D. P. F., Paris.

——— & J. A. H. Bakker-Heeres. 1985. Archaeobotanical studies in the Levant. I. Neolithic sites in the Damascus basin: Aswad, Ghoraife', Ramad. Plaeohistoria 24: 165–256.

———& S. Bottema. 1982. Vegetational history of the eastern Mediterranean and the Near East during the last 20,000 years. *In*: J. L. Bintliff & W. van Zeist (editors), Palaeoclimates, Palaeoenvironments and Human Communities in the Eastern Mediterranean Region in Later Quarternary. BAR Int. 133(2): 277–321.

Zohary, D. 1984. Modes of evolution under domestication. Pp. 579–586 *in* Plant Biosystematics. Academic Press, Canada.

——— & M. Hopf. 1973. Domestication of pulses in the Old World. Science 182: 887–894.

THE BENEFITS AND RISKS OF WOODY LEGUME INTRODUCTIONS

C. E. Hughes and B. T. Styles[1]

Abstract

Woody members of the Leguminosae are being planted as exotics on an unprecedented scale in all but a few tropical countries. Legumes are well suited to planting on harsh, degraded sites being fast-growing, readily established and managed, and often nitrogen-fixing. They provide such products as fuelwood and livestock fodder to rural communities and play a leading role in agroforestry and in reforestation efforts to rehabilitate degraded lands, control soil erosion, and prevent desertification. Woody legume introductions have turned weedy in places, however, necessitating expensive control programs. Additional drawbacks can be poor performance and low species acceptability. Some woody legumes exhibit particularly aggressive adaptively weedy combinations of frequent early flowering, successful seed dispersal, and seed longevity. Such aggressive species likely to become weeds are actively sought and used in reforestation. It is shown that, although the biology of a species may determine its potential weediness, the perception of a plant as a weed vs. as an asset is controlled by a combination of site and socioeconomic factors and land management practices. A more rational approach to introductions through consideration of biological and site factors and management is outlined, and ideas for indices of weediness hazard and introduction potential are discussed. Lack of knowledge prior to introduction often presents an obstacle to implementing these ideas. Some concrete recommendations of immediate practical importance are given.

I. Introduction

Woody legumes are being planted outside of their native ranges on an unprecedented scale, and the rate of spread of woody legume germplasm is rapidly accelerating (Turnbull, 1983). Since the 1950s most tropical and subtropical tree planting has concentrated on species suitable for industrial end uses, such as saw timber and pulp. Large-scale plantation establishment based mainly on a small number of genera *(Pinus, Eucalyptus, Tectona, and Gmelina)* has overshadowed other forestry activities. Few leguminous trees meet industrial requirements and, with some exceptions, woody legumes have been little used in the past.

However, following the growing realization of the severity of fuelwood shortages and environmental degradation in many tropical and subtropical areas (Arnold & Jongma, 1978; Eckholm, 1976; Grainger, 1982), the last decade has seen a rapid expansion of tree planting to meet nonindustrial needs of rural communities and to support agricultural systems in agroforestry situations, to revegetate degraded lands, and to protect areas from soil erosion and desertification. Legumes are at the forefront of this new phase of tree planting, and floras are being explored, species selected, and seed collected and distributed widely throughout the tropics. For example, the seed collections described by Hughes & Styles (1984) have now been distributed to 42 different tropical countries (Hughes, 1985). Appendix I lists woody legumes being planted worldwide, giving details of ecozones, countries, and end uses.

Most developing countries have or are initiating programs of social and community forestry with arborescent legumes as the mainstays (for an example from India, see Fernandes & Kulkarni, 1983). Several international bodies are promoting the use of woody legumes, e.g., the Food and Agriculture Organisation of the United Nations (FAO), the National Academy of Sciences (NAS),

[1]Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, England.

C. H. Stirton & J. L. Zarucchi (eds.)—Advances in Legume Biology
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the Nitrogen Fixing Tree Association (NFTA), and the International Council for Research in Agroforestry (ICRAF). Tree planting in rural development projects now lies at the core of the policies of some aid donors, e.g., World Bank, and large sums of money are now being invested in such programs. Planned United States Agency for International Development spending on fuelwood research alone stands at $76 million for 1983–1993 (Agency for International Development, 1983).

In the past few decades invasion by woody legume weeds has necessitated many expensive eradication or control programs. It is reported that *Mimosa pigra*, an infrequent woody shrub native to Central and South America, now covers over 10,000 hectares of farmland in northern Thailand and more than 5,000 hectares of river floodplain in northern Australia (Miller et al., 1981; Miller, 1982) with continued spread that is too expensive to control. In South Africa several woody legumes have been classified as noxious invaders (Stirton, 1983). *Prosopis ruscifolia* is reported to have invaded millions of hectares of natural pasture outside its natural range in the Chaco region of Argentina at considerable cost to livestock production (D'Antoni & Solbrig, 1977), while in Texas alone, Fisher (1977) reported that 1.2 million hectares of native *Prosopis glandulosa* var. *glandulosa* are treated annually with herbicides or mechanical equipment. Woody legumes are prominent elements of weed floras in many countries (Wells et al., 1983a; Kleinschmidt & Johnson, 1977) and may be threatening to contaminate the few remaining areas of natural undisturbed vegetation (Janzen, 1986; Stirton, 1983).

Clearly the accelerated rate of introduction of woody legumes raises the specter of greater weediness problems in the future. In many cases their introduction is controversial with pressure groups working on both sides, giving rise to debate, conflict, and many contradictory situations. While an estimated $1.5 billion are spent each year in the southern U.S.A. on mesquite (*Prosopis* spp.) control of which first became an issue over 50 years ago (Young et al., 1948), some range managers advocate its management to maintain a woody cover (Maltsberger, 1982). Although *Acacia mearnsii* has been labeled a "green cancer" of South Africa, A.C.A.C.I.A (A Case Against Controlling Introduced Acacias) is actively fighting its control (Stubbings, 1977) and the species is being introduced and promoted for planting throughout the tropical highlands (NAS, 1980; NFTA, 1985). Even for *Mimosa pigra*, where weediness would seem to be clearcut, several authors mention beneficial effects of planting (e.g., Suwignyo & Wiroatmodjo, 1982).

As far as we know, there are no comprehensive reviews of weediness in relation to woody legume introductions, and given the controversy surrounding the issue, it is hoped that this paper will make a timely contribution to the debate. In this paper the scope and importance of woody legumes both as useful trees and as weeds will be outlined; the factors influencing their performance as exotics will be discussed and alternatives for a more rational approach than the present unsatisfactory procedures for their introduction to new environments will be developed.

II. Benefits of Woody Legume Introductions

Over the past decade a growing realization has emerged of the contributions that forests and trees make directly and indirectly to rural populations in developing countries. In the past this contribution has been substantially underestimated by development workers. Trees provide fuelwood, livestock fodder, food, construction materials, posts and many minor products, such as weaving and thatching materials, tannins, medicines, gums, and bee forage. Woody legumes provide most of these, and their importance for fodder has been substantiated by Robinson's (1984) review of the chemical composition of fodder trees. Crane et al. (1984) showed that 79 of 452 important world honey sources are woody legumes. They also play a vital role in support of many traditional farming systems through maintenance of soil fertility in bush fallow systems and provision of dry-season fodder in silvopastoral systems or green manure and mulch in cropping systems. The value of agroforestry systems, combining trees and crops or livestock on the same area of land, is now well

appreciated. In arid zones trees may supply the only source of livestock feed or human food in drought years. Through protection of soil cover and amelioration of wind, temperature, and water runoff, trees help prevent soil erosion and land degradation. Evidence for the importance of these contributions has been discussed elsewhere (FAO, 1978, 1983; Eckholm, 1976).

FAO (1981) has determined that nearly 2,000 million hectares of tropical land have potential for reforestation, with deforested watersheds and desertified lands contributing 1,600 million hectares. Large areas of heavily eroded, degraded lands are in need of revegetation, protection, and rehabilitation, with most tree planting carried out on marginal or degraded sites associated with shallow, heavily leached soils following abandonment of agriculture. Climates are often severe, often with low rainfall or an extended dry season.

With such difficult conditions, reforestation efforts have concentrated almost entirely on aggressive colonizers well adapted to rapid establishment and growth on infertile, highly disturbed areas, e.g., species of *Pinus*, *Eucalyptus*, *Casuarina*, and *Alnus*. Species of these genera occur naturally on open disturbed areas, such as littoral, streambank, landslide, and tree-fall sites or as in fire-maintained climax vegetation. They are often found in pure stands or mixed with a few other similar species, e.g., tropical pines in Central America or *Eucalyptus* in Australia. Many woody legumes are robust colonizers and can occur in uniform pure stands (e.g., *Parkinsonia aculeata* on former lake bed sites or river floodplains in Central America (Fig. 1D) or *Gliricidia sepium* on coastal dunes in southern Mexico), making them particularly well adapted to reforestation on similar sites.

With few exceptions, tree planting programs have relied on exotic species, which—at least in the short term—almost invariably grow more vigorously than indigenous trees. In Rajasthan, India, for instance, *Acacia tortilis* and *Prosopis juliflora* outperform a range of local species (Figures 1A, 2B) (Muthana, 1982; Muthana & Arora, 1979, 1983). This is usually attributed to their escape from pests, diseases, and herbivorous predators. Many outstanding exotics are poorly known and little used or recognized within their native areas, e.g., *Pinus radiata* and other subtropical pines planted in the southern hemisphere, many species of *Eucalyptus* planted pantropically, and several legumes, such as *Calliandra calothyrsus*, which is extensively planted in Java but was virtually unknown within its native range in Central America until its success as an exotic was established (National Academy of Sciences, 1983a).

Many woody legumes are outstanding for their multiple uses and combination of benefits. *Leucaena leucocephala* (Pound & Martínez, 1983), *Gliricidia sepium* (Falvey, 1982), and *Calliandra calothyrsus* (NAS, 1983a) all provide fuelwood, livestock fodder, green manure, and bee forage (Fig. 2C). In a set of species lists of potentially valuable multipurpose tree species compiled by Burley (1983) from a variety of sources from different parts of the world, approximately 50% of the 2,000 taxa cited were legumes. Of the 25 species selected from the dry-zone flora of Central America by Hughes & Styles (1984) for fuelwood and other uses, 21 are legumes.

The ability of some legumes to fix atmospheric nitrogen has promoted their suitability for planting on nutrient-deficient sites and probably accounts for their success and natural abundance in dry tropical environments with characteristically infertile soils (Felker, 1981). Several authors have doubted the significance of nitrogen fixation. Despite the fact that Wigston (1985) has shown that many nitrogen-fixing species may make a net nitrogen demand on the soil during their period of rapid juvenile growth, this demand is likely to be greatly reduced compared with non-nitrogen-fixers. Sprent (1985) cited the case of woody legumes under regular lopping regimes as a case where nitrogen fixation is especially effective. Woody legumes have been used for green manure or mulch to replace inorganic nitrogen fertilizers in cropping systems. Kang et al. (1981, 1985) showed that alley cropping of *Leucaena leucocephala* can support maize production levels at 2 tons/ha or approximately 80% of the yield with N-fertilizer. *Gliricidia sepium* has been used in the same way (Sumberg, 1986) and also as green manure on rice paddies in India and Sri Lanka (Falvey, 1982). *Inga*, *Erythrina*, and *Gliricidia* are all used as shade trees over perennial crops, such as coffee and cocoa, with beneficial nitrogen inputs (Lindblad & Russo, 1986; Glover & Beer, 1986). In a similar

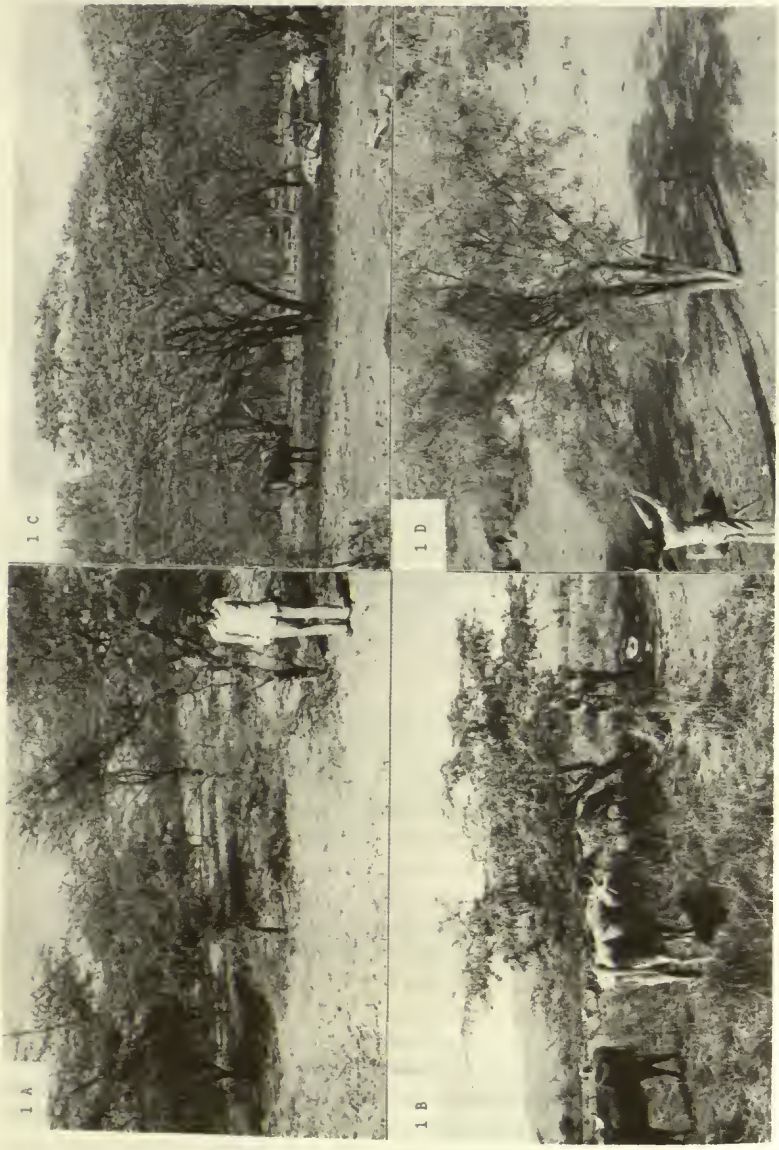

FIGURE 1.—A. *Acacia tortilis*, introduced to India from Israel in 1962, successfully planted for sand dune stabilization in Rajasthan. Nine years old.—B. *Prosopis juliflora* in its native range in Comayagua, Honduras, Central America, exhibiting unpalatable leaves and edible pods.—C. *Acacia pennatula* in northern Nicaragua, a useful species for fuelwood and pod production, with seed dispersal by livestock.—D. *Parkinsonia aculeata*, a prominent colonizer from Central America, widely planted in arid zones. Tree showing heavy pod production, Manabi, Ecuador.

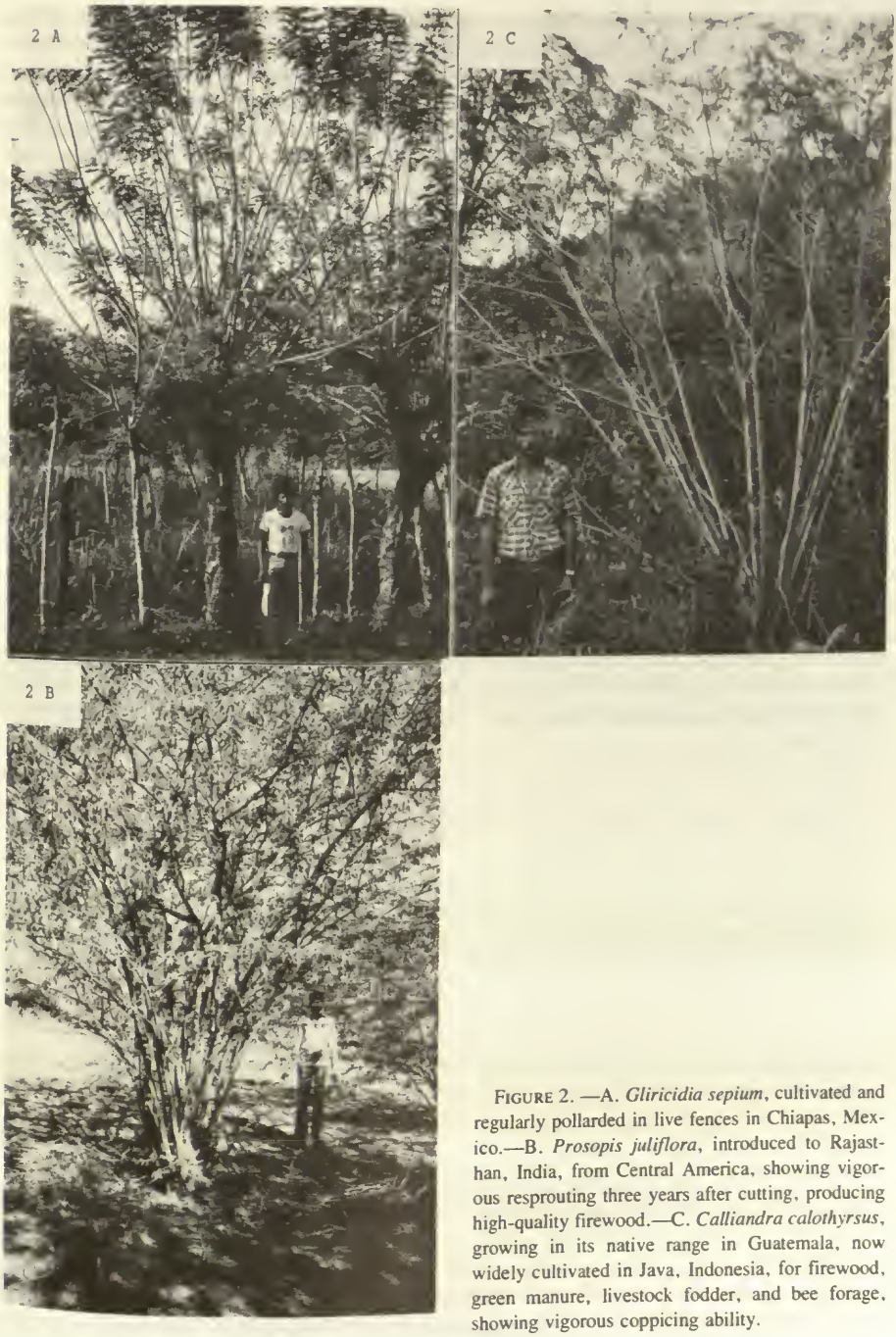

FIGURE 2. —A. *Gliricidia sepium*, cultivated and regularly pollarded in live fences in Chiapas, Mexico.—B. *Prosopis juliflora*, introduced to Rajasthan, India, from Central America, showing vigorous resprouting three years after cutting, producing high-quality firewood.—C. *Calliandra calothyrsus*, growing in its native range in Guatemala, now widely cultivated in Java, Indonesia, for firewood, green manure, livestock fodder, and bee forage, showing vigorous coppicing ability.

way inclusion of a woody legume component, such as *Albizia falcataria*, has been shown to boost growth of *Eucalyptus* in Hawaii (Debell et al., 1985).

Many woody legumes grow extremely rapidly and, although reliable yield estimates are hard to find (being largely based on early results from small unreplicated trials), several legume species are reportedly among the fastest-growing trees known, e.g., *Acacia mangium*, *Leucaena leucocephala*, and *Albizia falcataria*. Most of the yield data are based on small plots with large edge effects. The difficulty of assessing yields of sprawling, branchy trees compared with monopodial species has also made estimates of yield less reliable. Legumes frequently coppice and pollard vigorously, are readily propagated, and are easily managed. Ease of management by lopping makes them particularly suitable for use by farmers, e.g., use of *Gliricidia sepium* as live fences in Central America (Otarola et al., 1985).

Some legumes show considerable tolerances to extremes of heat, drought, and soil salinity. Felker (1981) cited the example of *Prosopis glandulosa* var. *torreyana* in Death Valley, California, with mean annual rainfall less than 70 mm and July daily maximum temperature close to 45°C. *Prosopis tamarugo* in the Atacama Desert of northern Chile tolerates similar conditions (Stienen, 1985). In many cases aggressive species that thrive on these sites have been sought by seed collectors in the expectation that most planting sites are similarly harsh (Figs. 1A, D) (Hughes & Styles, 1984; Boland & Turnbull, 1981).

Some legume species can be established despite heavy grazing because they exhibit a combination of unpalatable leaves (frequently with spines or toxic chemicals—Rehr et al., 1973) and highly nutritious edible pods (Felker et al., 1984; Odoul et al., 1986). Good examples are *Prosopis juliflora*, *Acacia pennatula*, *Enterolobium cyclocarpum* (Janzen, 1982a), and *Pithecellobium saman* (Janzen, 1982b). Such species have been successfully established in heavily grazed environments, e.g., *Prosopis juliflora* in Rajasthan, India (Muthana & Arora, 1983) and *Acacia tortilis* and *Prosopis chilensis* in Sudan (El Houri Ahmed, 1986), where establishment of vegetation cover can be achieved without excluding livestock and can still provide some benefit to the livestock through pod production (Fig. 1A, B, C).

III. Risks of Woody Legume Introductions

The introduction of exotic woody legumes has not always been beneficial. The recent spread of legume germplasm is likely to have associated with it a number of risks, if careful consideration of the biology of species, environment, socioeconomic, and management factors have not been undertaken before planting. The risks of introductions fall into two categories: (A) weediness and (B) poor site adaptation and species acceptability.

WEEDINESS

By far the most severe risk is weediness, and there are a growing number of expensive programs to control the spread of introduced, noxious, weedy legumes.

Weeds may be broadly defined as plants that interfere with man's activities, objectives, or requirements—*i.e.*, plants growing where they are not wanted. The objective definition of what is a weed is difficult and has been discussed elsewhere (Holzner, 1982; Harland & de Wet, 1965) but it is clear that it is impossible simply to label a given plant species as a weed. The perceived weediness of plant species differs from area to area, from person to person, and from year to year. The same species may be considered as harmless, as weedy, or as useful, depending on the place, time, and perception of the observer. Or, as Harlan & de Wet (1965) put it "one man's weed is another man's crop."

Weeds may also be defined in terms of their biology and ecological behavior. Although plants

defined in ecological terms as secondary succession colonizers may often become weeds, it is nevertheless wrong simply to equate weeds with pioneer species. Several known weeds do not fall into that category and many colonizers are not weeds. It is only through consideration of a plant's competition with man that weediness can be clearly defined (Holzner, 1982).

Many national and regional lists of weeds have been compiled, and some countries now maintain such weed lists. Based on these lists and on communications from weed scientists, a global list has been compiled by Holm et al. (1979) with a sublisting of the world's worst weeds (Holm et al., 1977). Appendix II, which lists all known woody legumes that have been classified as weeds, is largely based on Holm et al. (1979) but includes additions from other sources as indicated. Clearly this is by no means complete since many sources will have been missed and many instances of weediness are not documented. It does, however, indicate the scope of the problem and the range of genera involved.

The number of plants designated as weeds is expanding rapidly largely due to human activity. This has given rise to several useful classifications of weeds based on land management types (Holzner, 1982; Wells et al., 1983a, b) including:

a Ruderal—weeds of waste ground and disturbed habitats
b Agrestal—weeds of crops, orchards, and gardens
c Pastoral—weeds of grasslands
d Silvicultural—weeds of forestry plantations
e Floral/environmental—naturalized exotics that compete with the indigenous flora
f Health-related—weeds that harbor pests and diseases or act as alternate hosts of pathogens or insect pests
g Aquatic

Woody legume weeds occur in all these categories apart from truly aquatic habitats, although they are found on seasonally flooded shorelines, for example, *Mimosa pigra* in Australia and Thailand and *Albizia lophantha* in South Africa. Some appear in several categories but are particularly prominent as pastoral, silvicultural, ruderal, and floral weeds. These categories are discussed in detail below. Weeds may be exotic or indigenous, and, although this discussion is primarily concerned with introduced species, most weeds are in fact indigenous. The only category filled exclusively with exotics is floral or environmental weeds.

a. *Ruderal weeds*. These conform most closely to the ecological definition of weeds as colonizers in secondary succession. In this case highly disturbed areas of waste ground are invaded by a diverse group of plants with an increasing woody component as time passes.

b. Agrestal weeds. Woody legumes do not present major weed problems in arable situations where frequent intensive cultivation prevents their establishment, although a few exceptions do occur, e.g., *Mimosa pigra* in sugar cane plantations and rice paddies (Napompeth, 1982). In perennial crops, such as coffee, cocoa, and tea, where cultivation is less frequent and intensive, woody weeds are more common; a good example is *Leucaena leucocephala* when planted for shade over cocoa in Vanuatu and Hawaii.

c. *Pastoral weeds*. By far the greatest literature on woody legume weeds comes from range or pastoral situations in Australia, South Africa, southern U.S.A., a few African countries such as Zimbabwe and Uganda, and from the Chaco region of Argentina and Paraguay. In these areas exotics (e.g., *Acacia farnesiana*, *Prosopis glandulosa*, *Prosopis velutina*, and *Parkinsonia aculeata* in Australia) and indigenous species (e.g., *Prosopis glandulosa* in Texas, *Acacia hockii* in Uganda, *Mimosa tenuiflora* in Latin America (Fig. 3A), and *Prosopis ruscifolia* in Argentina) are important

FIGURE 3. —A. *Mimosa tenuiflora*, a pastoral weed native to Mexico, Central America, and northern South America, showing formation of dense, spiny thickets in Lara State, Venezuela.—B. *Caesalpinia pulcherrima*, a ruderal weed in Central America, growing on disturbed roadside sites, Choluteca, Honduras.—C. *Prosopis juliflora*, introduced to southern India from Central America, a ruderal weed of roadsides, gullies, and wastelands, and a vital source of firewood for poor people.—D. *Ulex europaeus*, gorse, a pastoral weed in western Scotland, showing dense, woody cover on former pasture land.

weeds. In all these cases large control or eradication programs have been undertaken with a heavy emphasis on broad spectrum herbicides. These pastoral weed situations are discussed in Section IV.

d. *Silvicultural weeds*. Although most forestry planting uses fast-growing aggressive colonizers, their early growth may frequently be affected by competition with other woody vegetation. Woody legumes are common weeds in subtropical pine plantations, e.g., *Acacia* species in *Pinus radiata* plantations in Australia (Flinn & Fagg, 1984), and are uncommon in temperate regions, where woody legumes are infrequent. In management of mixed natural forest, less desirable species are often regarded as weeds and are removed, e.g., *Robinia pseudoacacia* in the U.S.A. (Loftis, 1978). With expanding plantation programs in the tropics, where woody legumes are abundant, silvicultural weeds could assume greater importance in the future.

e. *Floral or environmental weeds*. These include plants that invade and compete with the indigenous flora. Close examination of the examples in this category shows that many of the so-called floral weeds are invading disturbed secondary formations (e.g., *Acacia melanoxylon* in South Africa (Geldenhuys, in press), *Leucaena leucocephala* in Vanuatu, *Albizia falcataria* in peninsular Malaysia, and *Robinia pseudoacacia* in seminatural pine-oak formations in south-central Europe). Invasion of disturbed sites or secondary vegetation broadly parallels colonization by ruderal weeds, and it is apparent that more highly disturbed sites are more susceptible to invasion by exotics than untouched areas. Clearcut examples of exotic woody legumes becoming elements of undisturbed pristine vegetation are few. The most striking examples come from South Africa, where several exotic woody legumes, including *Acacia cyclops*, *Acacia longifolia*, *Acacia mearnsii*, and *Sesbania punicea*, are actively invading the natural vegetation of the Cape Province (Stirton, 1983) as well as streambank and veld formations in the Transvaal (Henderson & Musil, 1984).

The idea that undisturbed natural vegetation is very resistant to invaders is reinforced by consideration of the numerous woody legume introductions that have failed, at least so far, to become elements of undisturbed ecosystems. In Central America no examples of woody legume invasion of biological reserves are known despite the introduction of several species that aggressively invade disturbed areas: *Caesalpinia pulcherrima*, (Fig. 3B) *Delonix regia*, *Leucaena leucocephala* (from southern Nicaragua southwards), *Cassia siamea*, and *Cassia fistula*. In South Africa Geldenhuys (in press) pointed out that only 14 of the 385 introduced trees and shrubs are able to spread into and establish successfully in undisturbed natural ecosystems. Janzen (1983, 1986) discussed interference with areas of pristine vegetation by means of invasion via surrounding natural disturbance sites (tree falls, riverbanks, coastal sites). With alteration of areas around a national park, Janzen (1983) showed that the species composition during succession on natural disturbance sites is greatly changed, and the ecological interactions involving predators, seed dispersal agents, and other factors may be greatly altered at least 5 km into the park. He also pointed out that native ruderal species will become more prominent in the disturbed secondary succession outside national parks which could alter canopy species composition within the park through time. Many remaining areas of undisturbed natural vegetation are extremely fragmented and small, and thus particularly susceptible to invasion (Geldenhuys et al., in press).

POOR SITE ADAPTATION AND SPECIES ACCEPTABILITY

Although widespread planting of woody legumes is in its infancy, sufficient experience has been gained to review progress and evaluate successes and failures. It is clear that a number of woody legumes have performed well as exotics and have been widely adopted for planting (see Appendix I). At the same time failures are apparent where large investments have been made with limited results in terms of areas of established trees. This is particularly apparent in the Sahel (World Bank, 1985; Taylor & Soumare, 1984), where an estimated $160 million was spent on forestry projects

involving woody legumes between 1975 and 1982 with very disappointing results. Low tree survival rates are probably attributable to many factors, including technical problems of plant propagation, use of untested, inappropriate germplasm, and the severity of the environment. It is also likely to be a function of the choice of species in relation to the perceived needs of the local people. Taylor & Soumare (1984) pointed to the exotics neem (*Azadirachta indica*) and mango (*Mangifera indica*) as examples of trees that have benefited from approval of local people who facilitated the rapid spread of the trees across West Africa. Similar examples are apparent from other areas, e.g., *Calliandra calothyrsus* in Java (NAS, 1983a) and *Tamarindus indica* in Central and West Africa. The view of tree planting as essentially a self-help process dependent on participation of local people was emphasized by Arnold (1984) and World Bank (1985). Failures are costly through wasted investment and in their impact on local communities who have frequently invested land and labor in planting and protecting the trees. Ill-advised species introductions can thus alienate local people and jeopardize future reforestation efforts.

IV. Factors Affecting Performance of Species in Exotic Environments and Their Potential to Become Weeds

The performance of an exotic plant species and man's perception of it as a weed or an asset depends on a range of biological characteristics and on a complex of interacting environmental, management, and socioeconomic factors associated with the area of introduction.

BIOLOGICAL FACTORS

The success of many exotics and the underlying causes of weediness are frequently attributed to absence of the indigenous herbivores and pathogens that attack plants in their native ranges. This has formed the basis of many successful introductions and biological control programs (Julien, 1982). But it is undoubtedly an oversimplification of a situation that is generally extremely complex, involving a series of factors that interact unpredictably, making the outcome of an introduction difficult to foresee. Nevertheless, it is valuable to consider the factors involved, and based on a few well-studied examples, it is possible to discern strong patterns and identify combinations of factors that promote weediness. The major biological factors influencing species performance are shown in Table 1.

A few of the factors relevant to the Leguminosae are discussed below.

Reproductive biology. This is of fundamental importance and can completely determine the fate of a species in a new environment and its tendency to weediness. A few woody legumes are subject to strict environmental control of flowering or seeding and have been widely used through vegetative propagation as successful exotics. These species rarely set seed and as a result do not spread,

Table 1. Biological factors affecting species performance in exotic environments.

Reproductive biology	flowering—plasticity, precocity, frequency, pollination
Seed production	abundance and frequency
Seed dispersal	animals, birds, water
Seed longevity and dormancy	
Vegetative reproduction	
Environmental tolerance	climate, soil, fire
Plant defense mechanisms	palatability, spininess, pests, diseases
Growth	rate, form, rooting habit, nitrogen-fixing ability

e.g., *Gliricidia sepium* in Sri Lanka or Malaysia. Most legumes, however, exhibit much less strict environmental control of flowering and seeding and are effectively pollinated by a wide range of insects (Simpson et al., 1977; Hunziker et al., 1986). Mimosoid species frequently have very low fruit/flower ratios, and it has been suggested that the very large number of flowers in many mimosoid genera serves to attract a wide range of insect pollinators to the frequently small and inconspicuous flowers (Solbrig & Cantino, 1975). For most legumes seed production is extremely abundant, which undoubtedly is a factor in their weediness, e.g., 95,000 seeds for *Mimosa pigra* per plant/year (Miller, 1982). Two other factors that promote rapid spread of a species are precocity and frequency of flowering. Many woody legumes flower during their first year (e.g., *Leucaena leucocephala*, *Gliricidia sepium*), and a few are able to flower either continuously (e.g., *Leucaena leucocephala*) or several times a year (e.g., *Mimosa pigra* and *Mimosa tenuiflora*) (Hughes, unpublished). Use of sterile forms and hybrids could provide a useful option to avoid weediness.

Escape from seed predation. In their native ranges, seeds of most legume species are heavily attacked by insect seed predators, mainly in the family Bruchidae. In many cases seed destruction rates are high, e.g., 93% for *Lysiloma acapulcense* in Honduras (Hughes & Styles, 1984) or 95% for *Ateleia herbert-smithii* in Costa Rica (Janzen, pers. comm.), and are usually at least 20% (Doran et al., 1983). Janzen (1980) and Johnson (1981) have shown the striking host specificity of seed beetles; many introduced legumes have escaped their seed predators. It has also been pointed out that habitat destruction by humans can reduce seed predation by disrupting the life cycles of predators, for example, *Bauhinia pauletia* in Costa Rica (Janzen, 1978).

Seed dispersal mechanisms. These have been implicated as of particular importance in plant invasion, with rapid spread by livestock of species with edible pods (Fig. 1C), e.g., *Prosopis* species (Mooney et al., 1977; Fisher, 1977; Glendening & Paulsen, 1955) and *Acacia* species (Chazaro Basanez, 1977). Edible pods provide good quality browse in many areas (Escalante, 1985), and livestock have been used in tree planting to promote seeding and tree establishment (El Houri Ahmed, 1986; Muthana & Arora, 1983). Long-distance seed dispersal by birds and water are also major factors in woody legume invasions. The spread of the Australian wattles *Acacia cyclops* and *A. melanoxylon*, planted in South Africa for sand dune stabilization and timber production, are examples of plant invasions promoted by bird dispersal of seeds (Stirton, 1983; Geldenhuys, in press). Seeds or light pod fragments of some legumes, e.g., *Mimosa pigra*, can be transported along water courses and across larger tracts of flooded land allowing particularly rapid movement into new areas. By contrast, legumes with unpalatable dehiscent pods and passive seed dispersal will spread much more gradually.

Seed longevity and dormancy. In control programs for woody legume weeds seed longevity has perhaps hindered progress more than any other factor. Legume seeds are generally long-lived due to their hard, impermeable seed coats (e.g., Martin, 1948, for *Prosopis* species; Le Roux, 1964, for *Acacia cyclops*). Glendening & Paulsen (1955) found good viability for seed of *Prosopis velutina* after 50 years storage in dry conditions. Miller et al. (1981) reported that *Mimosa pigra* plants are still reappearing in areas where control work has been actively carried out for more than 15 years and similar large soil-stored seed reserves have been documented for *Acacia melanoxylon* in South Africa (Geldenhuys, 1986).

Vegetative reproduction. Vegetative reproduction either by resprouts or root suckering is common in legumes, allowing them to tolerate extreme environmental conditions, fire, and browsing (Fig. 2B, C). These last two characteristics of seed longevity and vegetative reproduction highlight the extreme difficulty of eradicating a species exhibiting these features.

TABLE 2. Site and management factors affecting species performance in exotic environments.

Environmental	climate
	soil erosion and land degradation
Socioeconomic	population pressure from humans or livestock
	subsistence demand on woody vegetation
Management	agricultural production system
	size of unit
	intensity
	diversity in herd composition
	diversity of products
	multiple use of land

Environmental tolerance. The variable limits of environmental tolerance of woody legumes dictate the potential for spread. Species such as *Acacia melanoxylon* with a wide ecological amplitude in terms of soil and climate (Geldenhuys, in press) can invade very widely. When a species exhibits broad tolerances in all these traits it can be exceptionally aggressive and can invade rapidly, as in the case of *Mimosa pigra*, which has spread extensively in Thailand and northern Australia. Once established, a plant's performance depends on a complex set of factors controlling its competitive ability, including growth rate, tree form, rooting habit, nitrogen fixation, mycorrhizal associations, tolerance of environmental extremes, plant defense mechanisms, and versatility. It is not surprising that the very traits that make a species successful for tree planting in harsh degraded physical and biotic environments are those that promote invasiveness. Hence the same genera and often the same species are important in afforestation species and as major weeds (see Appendices I, II).

MANAGEMENT AND SITE FACTORS

Site factors that influence the potential weediness of a plant include environmental aspects of soil, climate, and vegetation, also socioeconomic conditions and management practices that may aggravate or alleviate weediness. (See Table 2.)

Socioeconomic factors. Of overriding importance are such socioeconomic factors as types of agricultural production systems and densities of humans and livestock. It is apparent in the progression from primitive hunter-gatherer societies through subsistence agriculture to intensive cash-cropping that dependence and pressure on woody vegetation decrease.

Examples of all stages of this sequence are visible even today within relatively small geographical areas. In Mexico a large proportion of the population consists of subsistence farmers, and it is estimated that 20% of the population depends on fuelwood. In areas of high population density, fuelwood is scarce and pressure on the woody vegetation is high (Evans, 1984). Trees also provide a vital source of food, livestock fodder, and construction materials in rural Mexico. Across the border, however, where extensive cattle ranching dominates in the southern U.S.A. in Texas and Arizona, woody vegetation, *Prosopis* species in particular, are largely recognized as weeds of rangeland (Glendening & Paulsen, 1955; Fisher, 1977). In these areas woody vegetation plays little or no part in the economy, is little used, and may be controlled.

Similar contrasts are apparent in other parts of the world; in many areas pressures on woody vegetation for local subsistence needs are so severe that reforestation is required, and weediness is not a hazard. In such situations success can only be assured by employing aggressive species, which have the potential to become weeds. Species acceptability and adoption by local populations will be partly determined by local requirements for wood products. In southern India, rural people are

entirely dependent on fuelwood for cooking. That there is not an acute shortage, and fuelwood collection is relatively easy, is entirely due to the presence of *Prosopis juliflora*, an aggressive invader introduced from Central America, which grows on all waste ground, roadsides, gullies, and pastures (Fig. 3C) (Hocking, pers. comm.; Muthana & Arora, 1983). In certain areas villagers say fuelwood is easier to get than it was a generation ago—thanks to *Prosopis juliflora*. Similar comments apply to other woody plants, such as *Lantana* species and *Eupatorium* species, widely classed as weeds. These so-called weeds are vital to the supply of fuel. For poor households such plants are particularly useful because of the very fact that they are not wanted by other sectors of the economy (Agarwal, 1985). Overall then, for large parts of the world and for the world's poorest communities in rural areas, woody weeds are not an issue.

Management practices. In areas where woody vegetation is not limited by human pressure the perception of a plant as a weed or an asset is largely determined by management practices. The intensity of management greatly affects weediness. Intensive, frequent cultivation normally prevents the establishment of woody weeds in arable situations. By far the greatest weed problems are from pastoral situations on large land holdings with low returns per unit area and low population densities. It is striking that the greatest research on weed control comes from seasonally dry or semiarid rangelands managed solely for meat production with cattle raised at low densities. Invasion of natural pasture or rangeland in these areas by woody legumes has been largely due to mismanagement with control of natural recurrent fires, over-stocking, and destructive selective grazing (Fisher, 1977; Wells & Stirton, 1982; Walker, 1980).

Excessive woody cover of undesirable species reduces palatable grass production and creates problems in mustering animals. It is now clear, however, that a certain level of woody cover is generally desirable through its beneficial influence on composition of grass species and through provision of valuable feed in browse and pods, particularly in the dry season when grass production is at a minimum (Fisher, 1977; Walker, 1980). Scifres et al. (1982) showed that 30% cover of *Acacia farnesiana* improved winter grass production in Texas and recommended a policy of bush management rather than bush control. As aridity increases, the importance of woody vegetation as a dry season fodder reserve or as a source of feed in drought years increases, and woody vegetation may provide up to 40% of total animal feed in North Africa (Le Houerou, 1980a) and 30% of animal intake in the Sahelian zone (Le Houerou, 1980a, b). Thus reduction in grass production can be compensated by secondary browse production, and the browse may be of better quality and available at times of year when grass production is at a minimum (Robinson, 1985). Clearly the relationship between woody vegetation and herbaceous cover is complex and will depend on a range of factors, including the density and growth form of the tree, the supply of available moisture, the soil type, and, most crucially, the management of the range. Alteration in management of the tree-pasture system can make the difference between tree cover being perceived as a weed or an asset. Any pasture situation with a drought-induced feed limitation will benefit from woody cover, the level of the optimal cover depending on tree species and herbivores.

Diversity in herd composition can lead to much greater exploitation of mixed rangeland and greater appreciation of woody browse than concentration on cattle alone. The complementary demands of different animals with cattle as grazers and goats as browsers can alter rangeland use, and diverse herds are an important part of the adaptive stategy of many nomadic pastoralists (Scott & Gormley, 1980). Strang (1973) discussed the benefits of goats and mixed game ranching over cattle for maintenance of weed-free pastures.

Diversity of products and multiple use of land also affect weediness. In these examples of rangeland management, meat is considered as the sole product with no consideration of wider benefits. Milk production and calving rates have often been neglected but may benefit from woody cover through provision of browse, pods, and shelter. Woody cover also provides charcoal and posts, is an important source of bee forage (Simpson et al., 1977), contributes nitrogen through biological fixation, and provides cover for wildlife. These wider benefits are rarely taken into

account when analyzing the profitability of woody weed control. Woody vegetation might not be seen as weedy if multiple use with diverse products was more prevalent.

Consideration of socioeconomic and management factors form the basis for development of a more rational approach to species introductions. They highlight the changing values of plants through time, from place to place, and from person to person. Plants currently considered as assets and actively introduced and promoted could, with changing circumstances, become weeds.

V. A Rational Approach to Species Introductions; Assessment of Weediness Hazard and Introduction Potential

PRESENT UNSATISFACTORY APPROACH

The present process of species introductions is characterized by a low level of concern about weediness hazards, little knowledge of the needs of the people the trees are designed to benefit, and uncontrolled distribution of germplasm around the world. The popularity of exotics is boosted by active promotion of a range of species by several international bodies and native species are often neglected. While most seed distribution agencies are aware of weediness hazards and typically include a warning in the accompanying information, e.g., NAS (1980, 1983b) or Hughes & Styles (1984), the seed recipient must decide on advisability of an introduction. Frequently warnings are overlooked or ignored. Many foresters are largely unaware of weediness issues that are primary concerns of range managers, national park managers, and other conservationists. Seed suppliers are remote from planting sites and cannot accurately assess weediness hazard; they can merely advise on biology and behavior of species in their native ranges.

As a result, few species are rejected due to weediness hazard. For example, Hughes & Styles (1984) cited *Mimosa tenuiflora* as extremely hazardous, yet only one country, Australia, out of 43 declined seed of that species. Seed was even requested by foresters in South Africa, where problems of weediness are severe and are relatively well documented and appreciated.

Many species are introduced casually through seed exchange between individuals and botanic gardens. Horticulturalists who introduce woody plants as ornamentals are major culprits causing problems of weediness with many cases of escaped introductions from gardens becoming major pests; *Lantana camara* and *Sesbania punicea* are examples. Wells et al. (1986b) pointed out that 42% of all South African naturalized exotics arrived as ornamentals. Although most exotic woody weeds have resulted from deliberate introductions, some accidental introductions have occurred, and some species have spread over long distances from naturalized populations or from the natural ranges.

THE RATIONAL APPROACH

The present unsatisfactory approach could be improved by a more careful study and consideration of the biology and potential weediness of species and by use of appraisal techniques to assess the likely impact or introduction potential of a species in a new area. This could be done by more careful matching of species to sites to avoid poor performance and by making introductions more selective in the light of local needs. Weediness index or species introduction potential must be an assessment of the environmental characteristics of the introduction site and of socioeconomic aspects, including management practices used in local farming systems.

It is notable that for the majority of woody legume introductions species biology is at best scantily studied prior to introduction, with very few complete autecological studies even of the better-known cultivated taxa. Such studies are often not undertaken until control measures are needed, e.g.,

Glendening & Paulsen (1955) for *Prosopis velutina*. Thus assessment of weediness hazard is greatly hampered by lack of basic knowledge about the species.

Many attempts have been made to develop methods to predict species performance in new environments based on identifying homoclinal locations. Booth (1985) described the bioclimatic prediction system, which is one of the most advanced approaches to this where the climatic environment of a species is assessed within its natural range; the results are used to define potential exotic locations for testing the species. This method has been applied successfully to *Eucalyptus citriodora* in Africa and could be more widely used to avoid inadvisable introductions.

Methods to assess such factors as fuelwood demand are generally available. For example, numerous surveys have been carried out in different areas to measure fuelwood use and likely shortages, e.g., Martínez (1982) for Guatemala. FAO (1983) has mapped the global fuelwood situation. Many areas of fuelwood shortage are extremely localized around urban centres, e.g., Managua, Nicaragua (Park et al., 1982). The diagnosis and design technique developed by ICRAF (1983) helps identify suitable inputs to production systems.

The scant attention paid to local needs in selecting species for tree planting has been implicated in the failure of many reforestation efforts (Arnold, 1984), as discussed in Section III. The blanket approach of testing all available species should now be modified. Careful assessment of the use and role of species in their native ranges through close contact and surveys of local wood users and farmers is a sound starting point now routinely used in many exploration and species selection projects (Hughes & Styles, 1984). A similar process must, however, be employed in the area of proposed introduction. Species introduction projects that are designed, and in many cases implemented and imposed, "from the top down," and dominated by the perceptions and priorities of planners and forestry technicians with little consideration of local needs are unlikely to succeed (Taylor & Soumare, 1984). In this way species introductions will be much more selective, and this will contribute to avoidance of weediness.

Where doubt about a species exists, it should be introduced initially on a small scale in carefully monitored species elimination trials that allow assessment of weediness hazard, site adaptability, and acceptability to local people prior to wider planting. If necessary, the species can be eradicated at an early stage. This has recently been recommended for *Mimosa tenuiflora* in the Oxford Forestry Institute's coordinated dry zone species trials.

Introductions are justified for the most difficult sites. However, even in extremely damaged areas, introduction of exotics should follow investigation and screening of indigenous species. Where pressures are less severe, rehabilitation and management of natural vegetation may provide viable alternatives.

VI. Discussion and Conclusions

On highly disturbed sites and severely degraded land, where the natural vegetation cover has been reduced beyond recovery, and in areas where subsistence demands on the woody vegetation are high, certain woody legumes are outstanding in their ability to survive, grow, and provide a range of products and services. In these areas it is unlikely that such species will be considered as weeds. If they contribute towards improving conditions for rural communities, and many examples already cited show that they can, then it is unacceptable to prevent their wider introduction. Such a limitation could greatly hamper many reforestation efforts in the critical areas where revegetation is most difficult.

Widespread introductions will, however, not necessarily be beneficial until a more stringent preliminary analysis is applied to predict their outcomes. The introduction of variable, often little-known genetic material into diverse, poorly understood environments is a highly complex process with the results difficult to forecast. The potential of a species to become a weed can be assessed on the basis of a range of biological characteristics as outlined above. However, since the same

characteristics are being sought in tree planting, it is much more important to examine the nature of the introduction site, since the site, socioeconomic factors, and management factors dictate the outcome of an introduction.

A more rational approach will greatly improve the present ad hoc situation, but several important limitations are apparent. Analysis is inevitably hampered by lack of knowledge. Few species are intensively studied prior to introduction, and in many cases introduction sites are poorly understood. Autecological studies of potential species would be of great value and should form an integral part of any introduction program. Similarly, very few introductions are carefully monitored and documented. Documentation of weediness case histories has been neglected, often being considered an unpopular subject, but is an important and essential ingredient in avoiding unwise introduction in the future.

More problematic is the mobility of plants once introduced to a new area. Since many areas where aggressive woody legumes might justifiably be introduced are limited in extent, escape to other adjacent areas where pressures are less severe could be detrimental and costly. For example, introduction of *Prosopis juliflora* to alleviate an acute fuelwood shortage around an urban center in Africa, such as Mogadishu in Somalia, could damage adjacent rangeland for nomadic pastoralists. Similarly, *Cassia siamea* cultivated for fuelwood around Managua, Nicaragua could alter seminatural or lightly disturbed seasonally dry vegetation in central Nicaragua or adjacent countries. In time, introductions will spread widely across whole continents into areas with widely varying environments, socioeconomic situations, and production systems.

Views about plants also change through time with changing technology, economics, and management, as seen in the example of gorse *(Ulex europaeus)*, which has changed from a widely cultivated winter feed bush to a pasture weed in the last century in the United Kingdom (Fig. 3D). A decline in tannin demand has greatly altered the value of *Acacia mearnsii* in South Africa. Thus even a rational approach to introductions cannot totally avoid risk of weediness when plants escape or circumstances change.

There is, however, a strong case for making woody legume introductions more selective. Although native species may be less productive than exotics and may cause severe weediness problems, they are generally already accepted and preferred by local people and should always be considered in reforestation. In less extreme situations, rehabilitation and management of natural vegetation is a valuable alternative.

We feel that clearing and mismanagement of vegetation by humans has had a much greater impact than have (or will) plant introductions. On the other hand, it is equally clear that the potential impact of plant introductions on the *existing* vegetation is extremely great. Likewise, introduced plants have a high potential for damage and relief in reforestation and other land use efforts. Certainly the introduction of exotics per se to highly disturbed sites does not generate weed problems, though such plants may later invade where they are unwanted.

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References

Agarwal, A. 1985. Human-nature Interactions in a Third World Country. 5th World Conservation Lecture. World Wildlife Fund.

Agency for International Development. 1983. Fuelwood Research Implementation Plan. A.I.D., Washington, D.C.

Arnold, J. E. M. 1984. Forestation for local community development. Pp. 48–62 *in* K. F. Wiersum (editor), Strategies and Designs for Afforestation, Reforestation and Tree Planting. Wageningen, the Netherlands.

——— & J. J. Jongma. 1978. Fuelwood and charcoal in developing countries: an economic survey. Unasylva 29(48): 2–9.

Boland, D. J. & J. W. Turnbull. 1981. The selection of Australian trees other than eucalypts for trials as fuelwood species in developing countries. Austral. Forest. 44: 235–246.

Booth, T. H. 1985. A new method for assisting species selection. Commonw. Forest. Rev. 64: 241–257.

Brewbaker, J. L. & B. T. Styles. 1983. Economically important NFT. *In*: Resource Documents on Nitrogen-Fixing Trees. Nitrogen-Fixing Tree Association, Waimanalo, Hawaii.

Burley, J. 1983. Global needs and problems of collection, storage and distribution of multipurpose tree germplasm—a background document. Pp. 43–221 *in* J. Burley & P. von Carlowitz (editors), Multipurpose Tree Germplasm—Proceedings of a Planning Workshop. ICRAF, Nairobi, Kenya.

Chazaro Basanez, Mo de J. 1977. El huizache, *Acacia pennatula* (Schlecht. and Cham.) Benth., Especie invasora del centro de Veracruz. Biotica 2(3): 1–17.

Cock, M. J. W. 1982. Report on a Consultancy to the Republic of Vanuatu to advise on biological control of weeds and pests. Commonw. Inst. Biological Control, Ascot, U.K. Unpublished report.

Crane, E., P. Walker & R. Day. 1984. Directory of Important World Honey Sources. International Bee Research Association, London.

D'Antoni, H. L. & O. T. Solbrig. 1977. Algorrobos in South American cultures: past and present. Pp. 189–200 *in* B.B. Simpson (editor), Mesquite: Its Biology in Two Desert Ecosystems. US/IBP Synthesis Series 4. Dowden, Hutchison & Ross, Pennsylvania.

Debell, D. S., C. D. Whitesell & T. H. Schubert. 1985. Mixed plantations of *Eucalyptus* and leguminous trees enhance biomass production. Research Paper PSW-175. Pacific Southwest Forest and Range Experiment Station, Forest Service, USDA.

Doran, J. C., J. W. Turnbull, D. J. Boland & B. V. Gunn. 1983. Handbook on Seed of Dry Zone Acacias. FAO, Rome.

Eckholm, E. P. 1976. Losing ground—environmental stress and world food prospects. Worldwatch Institute, U.S.A.

El Houri Ahmed, A. 1986. Some aspects of dry land afforestation in the Sudan with special reference to *Acacia tortilis*, *A. senegal* and *Prosopis chilensis*. Forest Ecol. & Managem. 16: 209–221.

Escalante, E. E. 1985. Promising agroforestry systems in Venezuela. Agroforestry Syst. 3: 209–221.

Evans, M. I. 1984. Firewood versus alternatives: domestic fuel in Mexico. Commonwealth Forestry Institute, Oxford, Occ. Pap. 23: 1–66.

Falvey, L. 1982. *Gliricidia maculata*—a review. Int. Tree Crops. J. 2: 1–14.

FAO. 1978. Forestry for local community development. Forestry Pap. No 7. FAO, Rome.

———. 1980. Genetic resources of tree species in arid and semi-arid areas. FAO, Rome.

———. 1981. Report of the Fifth Session of the FAO Panel of Experts on Forest Gene Resources. Rome.

———. 1983. Fuelwood supplies in the developing countries. Forestry Pap. No. 42. FAO, Rome.

Felker, P. 1981. Uses of tree legumes in semi-arid regions. Econ. Bot. 35: 174–186.

———. 1984. Economic, environmental and social advantages of intensively managed short rotation mesquite (*Prosopis* spp). Biomass energy farms. Biomass 5: 65–77.

———, P. R. Clark, J. F. Osborn & G. H. Cannell. 1984. *Prosopis* pod production—comparison of North American, South American, Hawaiian and African germplasm in young plantations. Econ. Bot. 38: 36–51.

Fernandes, W. & S. Kulkarni. 1983. Towards a new forest policy: people's rights and environmental needs. Indian Social Institute, New Delhi, India.

Fisher, C. E. 1977. Mesquite and modern man in southwestern North America. Pp. 177–188 *in* B. B. Simpson (editor), Mesquite: its biology in two desert ecosystems. US/IBP Synthesis Series 4. Dowden, Hutchison & Ross, Pennsylvania.

Flinn, D. W. & P. C. Fagg. 1984. Review of weed control practices in radiata pine plantations in Australia. *In*: R. W. Madin (editor), Proceedings of the Seventh Australian Weeds Conference, Volume 1. Melbourne, Australia.

Geldenhuys, C. J. Costs and benefits of the Australian Blackwood, *Acacia melanoxylon*, in South African forestry. *In*: I. A. W. Macdonald & F. J. Kruger (editors), The Ecology and Management of Biological Invasions in Southern Africa. Oxford Univ. Press, Cape Town, South Africa (in press).

———, P. J. Le Roux & K. H. Cooper. Alien invasions in indigenous evergreen vegetation. *In*: I. A. W. Macddonald & F. J. Kruger (editors), The Ecology and Management of Biological Invasions in Southern Africa. Oxford Univ. Press, Cape Town, South Africa (in press).

Glendening, G. E. & H. A. Paulsen. 1955. Reproduction and establishment of velvet mesquite as related to invasion of semidesert grasslands. U.S.D.A. Forest Service Tech. Bull. 1127: 1–50.

Glover, N. & J. Beer. 1986. Nutrient cycling in two traditional Central American agroforestry systems. Agroforestry Syst. 4(2); 77–88.

Grainger, A. 1982. Desertification. Earthscan, London.

Harlan, J. R. & J. M. J. de Wet. 1965. Some thoughts about weeds. Econ. Bot. 19: 10–24.

Harrington, G. N. 1983. Bush control: a note of caution. E. African Agric. & Forest. J. (in press).

Henderson, L. & K. J. Musil. 1984. Exotic woody plant invaders of the Transvaal. Bothalia 15: 297–313.

Henty, E. E. & G. S. Pritchard. 1973. Weeds of New Guinea and their control. Botany Bulletin No. 7. Department of Forests, Division of Botany, Lae.

Hodgkinson, K. C. 1983. Prescribed burning for woody weed control in semi-arid woodlands. *In*: Proc. Workshop on Woody Weeds of Northern Summer Rainfall Areas. Rockhampton, Queensland, Australia.

Holm, L. G., J. V. Pancho, J. P. Herberger & D. L. Plucknett. 1979. A Geographical Atlas of World Weeds. Wiley, New York.

———, D. L. Plucknett, J. V. Pancho & J. P. Herberger. 1977. The World's Worst Weeds: Distribution and Biology. East-West Centre, Univ. of Hawaii, Honolulu, Hawaii.

Holzner, W. 1982. Concepts, categories and characteristics of weeds. Pp. 3–20 *in* W. Holzner & M. Numata (editors), Biology and Ecology of Weeds. Junk, the Netherlands.

Hughes, C. E. 1985. Tropical Hardwoods for Dry and Arid Zones. Unpublished Report to ODA on Research Scheme R.3714. Overseas Development Administration, London.

——— & B. T. Styles. 1984. Exploration and seed collection of multipurpose dry zone trees in Central America. Int. Tree Crops J. 3(1): 1–31.

Hunziker, J. H., B. O. Saidman, C. A. Naranjo, R. A. Palacios, L. B. O. Poggio & A. D. Burghardt. 1986. Hybridisation and genetic variation of Argentine species of *Prosopis*. Forest Ecol. & Managem. 16: 301–315.

ICRAF. 1983. Guidelines for Agroforestry Diagnosis and Design. Working Paper No. 6. ICRAF, Nairobi, Kenya.

Ivens, G. W. 1967. East African Weeds and Their Control. Oxford Univ. Press, Nairobi, Kenya.

Janzen, D. H. 1974. Swollen-thorn acacias of Central America. Smithsonian Contrib. Bot. 13: 1–131.

———. 1978. Reduction of seed predation on *Bauhinia pauletia* (Leguminosae) through habitat destruction in a Costa Rican deciduous forest. Brenesia 14–15: 325–335.

———. 1980. Specificity of seed attacking beetles in a Costa Rican deciduous forest. J. Ecol. 68: 929–952.

———. 1982a. Differential survival and passage rates in cows and horses: surrogate Pleistocene dispersal agents. Oikos 38: 150–156.

———. 1982b. Cenizero tree (Leguminosae: *Pithcellobium saman*) delayed fruit development in Costa Rican deciduous forests. Amer. J. Bot. 69: 1269–1276.

———. 1983. No park is an island: increase in interference from outside as park size decreases. Oikos 41: 402–410.

———. 1987. The eternal external threat. Pp. 286–303 *in* M. E. Soulé (editor), Conservation Biology: the Science of Scarcity and Diversity. Sinauer Associates, Sunderland, Massachusetts.

Johnson, C. D. 1981. Seed beetle host specificity and the systematics of the Leguminosae. Pp. 995–1027 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. Royal Botanic Gardens, Kew.

Julien, M. H. 1982. Biological control of weeds: a world catalogue of agents and their target weeds. Commonwealth Agricultural Bureau.

KANG, B. T., G. F. WILSON & L. SIPKENS. 1981. Alley cropping of maize (*Zea mays* L.) and *Leucaena* (*Leucaena leucocephala*) in southern Nigeria. Pl. & Soil 63: 165–179.

———, H. GRIMME & T. L. LAWSON. 1985. Alley cropping sequentially cropped maize and cowpea with *Leucaena* on a sandy soil in southern Nigeria. Pl. & Soil 67: 267–277.

KLEINSCHMIDT, H. E. & R. W. JOHNSON. 1977. Weeds of Queensland. Queensland Dept. of Primary Industries, Brisbane, Australia.

LAMP, C. & F. COLLET. 1979. A Field Guide to Weeds in Australia. Inkata Press.

LE HOUEROU, H. N. 1980a. Browse in northern Africa. Pp. 55–82 *in* H. N. Le Houerou (editor), Browse in Africa—the Current State of Knowledge. International Livestock Centre for Africa, Addis Ababa, Ethiopia.

———. 1980b. The role of browse in the Sahelian and Sudanian zones. Pp. 83–100 *in* H. N. Le Houerou (editor), Browse in Africa—the Current State of Knowledge. International Livestock Centre for Africa, Addis Ababa, Ethiopia.

LE ROUX, E. R. 1964. The Australian acacias in South Africa. Pp. 137–142 *in* D. H. Davies (editor), Ecological Studies in Southern Africa. Junk, The Hague.

LINDBLAD, P. & R. RUSSO. 1986. C^2H^2-reduction by *Erythrina poeppigiana* in a Costa Rican coffee plantation. Agroforestry Syst. 4(1): 33–38.

LIOGIER, A. H. 1969. Flora de Cuba, Supplemento.

LOFTIS, D. L. 1978. Preharvest herbicide control of undesirable vegetation in southern Appalachian hardwoods. Southern J. Appl. Forest. 2(2): 51–54.

MALTSBERGER, W. 1982. Bill Maltsberger boosts grazing on large ranch fenced in cells. Livestock Weekly 1982: 11.

MARTIN, S. C. 1948. Mesquite seeds remain viable after 44 years. Ecology 29: 393.

MARTINEZ, A. H. 1982. Estudio sobre leña en hogares, pequeña industria y distribuidores de Guatemala. Proyecto CATIE/ROCAP No. 596-0089 Informe Tecnico 27, CATIE Turrialba, Costa Rica.

MILLER, I. L. 1982. The distribution and threat of *Mimosa pigra* in Australia. Pp. 38–50 *in* Proceedings of an International Symposium on *Mimosa pigra* management. Chiang Mai, Thailand. International Plant Protection Center, Corvallis, Washington. 48-A-83.

——— & S. E. PICKERING. 1980. *Parkinsonia*: a noxious weed. Northern Territory, Dept. of Primary Production, Darwin, Australia. Agnote 80/37.

———, L. NEMESTOTHY & S. E. PICKERING. 1981. *Mimosa pigra* in the Northern Territory. Dept. Primary Production, Division of Agriculture, Darwin, Australia Techn. Bull. 51: 1–23.

MOONEY, H. A., B. B. SIMPSON & O. T. SOLBRIG. 1977. Phenology, morphology and physiology. Pp. 26–43 *in* B. B. Simpson (editor), Mesquite: Its Biology in Two Desert Ecosystems. US/IBP Synthesis Series 4. Dowden, Hutchison & Ross, Pennsylvania.

MOORE, R. M. 1971. Weeds and weed control in Australia. J. Australian Inst. Agric. Sci. 37(3): 181–191.

MUTHANA, K. D. 1982. Selection of tree species in arid zone forestry. Pp. 103–111 *in* P. K. Khosla (editor), Improvement of Forest Biomass. Indian Society of Tree Scientists.

——— & G. D. ARORA. 1979. *Acacia tortilis*—a promising tree for Indian arid zones. Techn. Bull. No. 5. CAZRI, Jodhpur, India.

——— & ———. 1983. *Prosopis juliflora* (Swartz) DC., a fast growing tree to bloom the desert. CAZRI Monogr. 22: 1–21.

NAPOMPETH, B. 1982. Background, Threat and Distribution of *Mimosa pigra* in Thailand. Proceedings International Symposium *Mimosa pigra* Management. Chiang Mai, Thailand. International Plant Protection Center, Corvallis, Washington. 48-A-83.

NATIONAL ACADEMY OF SCIENCES. 1980. Firewood Crops: Shrub and Tree Species for Energy Production, Volume 1. NAS, Washington, D.C.

———. 1983a. *Calliandra* a versatile small tree for the humid tropics. Innovations in Tropical Reforestation Series. NAS, Washington, D.C.

———. 1983b. Firewood Crops: Shrub and Tree Species for Energy Production, Volume 2. NAS, Washington, D.C.

NITROGEN FIXING TREE ASSOCIATION. 1985. *Acacia mearnsii*—multipurpose highland legume tree. NFT Highlights 85-02.

ODOUL, P. A., P. FELKER, C. R. MCKINLEY & C. E. MEIER. 1986. Variation among selected *Prosopis* families for pod sugar and pod protein contents. Forest Ecol. & Managem. 16: 432–431.

OTAROLA, A., H. MARTINEZ & R. ORDONEZ. 1985. Manejo y producción de cercas vivas de *Gliricidia sepium* en el noroeste de Honduras. CATIE, Turialba, Costa Rica.

Park, W., L. C. Newman & K. Ford. 1982. Fuelwood supply for Managua Nicaragua—sustainable alternatives for the Las Maderas Fuelwood Supply Region. Mitre Corporation for CATIE/ROCAP.

Parsons, W. T. 1973. Noxious Weeds of Victoria. Inkata Press, Australia.

Pound, B. & L. C. Martinez. 1983. *Leucaena*, Its Cultivation and Uses. Overseas Development Administration, London.

Rehr, S. S., P. Feeny & D. H. Janzen. 1973. Chemical defences in Central American non-ant acacias. J. Anim. Ecol. 42: 405–416.

Roberts, H. A. (editor). 1983. Weed Control Handbook: Principles, 7th edition. British Crop Protection Council. Blackwell, Oxford.

Robinson, P. J. 1984. Data base on fodder tree chemical composition and feed value. Nitrogen-Fixing Tree Res. Rep. 2: 26–27.

———. 1985. Trees as Fodder Crops. Pp. 281–300 *in* M. G. R. Cannell, J. E. Jackson & J. C. Jordon (editors), Attributes of Trees as Crop Plants. ITE, United Kingdom.

Scifres, C. J., J. L. Mutz, R. E. Witson & D. L. Drawe. 1982. Interrelationships of Huisache canopy cover with range forage on the coastal prairie. J. Range Managem. 35: 558–562.

Scott, M. F. & B. Gormley. 1980. The animal of friendship (Habbanaae): an indigenous model of Sahelian pastoral development in Niger. Pp. 93–112 *in* D. W. Brokensha, D. M. Warren & O. Werner (editors), Indigenous Knowledge Systems and Development. Univ. Press of America, Lanham, Maryland.

Simpson, B. B., J. L. Neff & A. R. Moldenke. 1977. *Prosopis* flowers as a resource. Pp. 84–107 *in* B. B. Simpson (editor), Mesquite: Its Biology in Two Desert Ecosystems. US/IBP Synthesis Series 4. Dowden, Hutchison & Ross, Pennsylvania.

Solbrig, O. T. & P. D. Cantino. 1975. Reproductive adaptations in *Prosopis* (Leguminosae, Mimosoideae). J. Arnold Arboretum 56: 185–210.

Sprent, J. L. 1985. Nitrogen fixation in arid environments. Pp. 215–229 *in* G. E. Wickens, J. R. Goodin & D. V. Field (editors), Plants for Arid Lands. Royal Botanic Gardens, Kew.

Stienen, H. 1985. *Prosopis tamarugo* in the Chilean Atacama—ecophysiological and reforestation aspects. Pp. 103–116 *in* G. E. Wickens, J. R. Goodwin & D. V. Field (editors), Plants for Arid Lands. Royal Botanic Gardens, Kew.

Stirton, C. H. 1983. Plant Invaders: Beautiful but Dangerous. Dept. Nature and Environmental Conservation of the Cape. Provincial Administration, Cape Town, South Africa.

Strang, R. M. 1973. Bush encroachment and veld management in south-central Africa: the need for a reappraisal. Biol. Conservation 5(2): 96–104.

Stubbings, J. A. 1977. A.C.A.C.I.A. S. African Forest. J. 102: 8–14.

Sumberg, J. E. 1986. Collection and initial evaluation of *Gliricidia sepium* from Costa Rica. Agroforestry Syst. 3(4): 357–361.

Suwignyo, P. & J. Wiroatmodjo. 1982. Role and function of *Mimosa pigra* in reservoirs. Pp. 51–55 *in* Proceedings of an International Symposium on *Mimosa pigra* Management. Chiang Mai, Thailand. International Plant Protection Center, Corvallis, Washington. 48-A-83.

Taylor, G. F. & M. Soumare. 1984. Strategies for forestry development in semi-arid tropics—lessons from the Sahel. Pp. 137–167 *in* K. F. Wiersum (editor), Strategies and Designs for Afforestation, Reforestation and Tree Planting. Pudoc Wageningen, the Netherlands.

Terry, P. J. 1982. Appraisal of weed problems in the Republic of Vanuatu, 28 February–14 March 1982. ARC, Weed Research Organization. Internal Report No. 149: 1–26.

———. 1983. Some Common Crop Weeds of West Africa and Their Control. USAID, Dakar, Senegal.

Turnbull, J. W. 1983. Tree seed supply—a critical factor for the success of agroforestry projects. Pp. 279–298 *in* J. Burley & P. von Carlowitz (editors), Multipurpose Tree Germplasm–Proceedings of a Planning Workshop. ICRAF, Nairobi, Kenya.

Walker, B. H. 1980. A review of browse and its role in livestock production in southern Africa. Pp. 7–24 *in* H. N. Le Houerou (editor), Browse in Africa—the Current State of Knowledge. International Livestock Centre for Africa, Addis Abba, Ethiopia.

Wells, M. J. & C. H. Stirton. 1982. Weed problems of South African pastures. Pp. 429–433 *in* W. Holnzer & M. Numata (editors), Biology and Ecology of Weeds. Junk, the Netherlands.

———, V. M. Engelbrecht, A. A. Balsinhas & C. H. Stirton. 1983a. Weed flora of South Africa 1: major groupings. Bothalia 14: 945–948.

———, ———, ——— & ———. 1983b. Weed flora in South Africa 2: power shifts in the veld. Bothalia 14: 961–965.

———, A. A. Balsinhas, H. Joffe, V. M. Engelbrecht, G. Harding & C. H. Stirton. 1986a. A catalogue of problem plants in southern Africa. Memoirs of the Botanical Survey of South Africa (in press).

Wells, M. J., R. J. Poynton, A. A. Balsinhas, K. J. Musil, H. Joffe, E. van Hoepen & S. K. Abbot. 1986b. The history of introduction of invasive alien plants to southern Africa via sea and air routes (in press).

Wigston, D. L. 1985. Nitrogen demand and nitrogen fixation in woody plants—cautionary tale. Klinkii 3(1): 52–69.

World Bank. 1985. Desertification in the Sahelian and Sudanian Zones of West Africa. World Bank, Washington, D.C.

Young, V. A., F. R. Anderwald & W. G. McCully. 1948. Bush problems on Texas ranges. Texas Agric. Exp. Station Miscellaneous Publication 21: 1–19.

Appendix I. Woody legumes of widespread importance in reforestation. Sources: Brewbaker & Styles (1983); National Academy of Sciences (1980, 1983b); FAO (1980).

Species	Ecozone HT—Humid Tropics ASA—Arid/ Semiarid TH—Tropical Highlands	Countries (Main countries or regions of use)	End uses: Fuelwood	Livestock fodder	Green manure	Poles and posts	Erosion control	Other
Acacia albida	ASA	Africa-Sahelian	X	X	X	X	X	
A. aneura	ASA	Africa, Australia		X			X	
A. auriculiformis	HT	Indonesia, Malaysia	X			X		pulp
A. brachystachya	ASA	Australia	X	X		X		
A. cambagei	ASA	India, West Africa	X			X		wood
A. confusa	HT	Philippines	X					
A. cyclops	ASA	South Africa	X				X	
A. farnesiana	ASA	Pantropical	X	X			X	
A. mangium	HT	Southeast Asia	X			X		
A. mearnsii	TH	Southern and Eastern Africa, India	X		X		X	tannin
A. nilotica	ASA	Africa, India	X	X	X	X	X	
A. saligna	HT (ASA)	South Africa	X	X		X	X	
A. senegal	ASA	Africa, Pakistan, India	X	X		X	X	gum
A. seyal	ASA	Africa	X	X		X		gum
A. tortilis	ASA	India	X	X		X	X	
Albizia caribaea	ASA (HT)	Sri Lanka	X			X	X	
A. falcataria	HT	Philippines	X					pulp
A. lebbek	ASA	India, Africa	X	X		X	X	
A. procera	ASA	India	X	X		X		
Cajanus cajan	ASA	Pantropical	X	X	X		X	food
Calliandra calothyrsus	HT	Indonesia	X		X		X	
Cassia siamea	ASA (HT)	West Africa, Central America	X		X	X	X	
Dalbergia sissoo	ASA	West Africa, India	X				X	
Derris indica[1]	HT (ASA)	Pantropical	X	X		X		
Erythrina fusca	HT	South and Central America				X		shade
E. poeppigiana	HT	South and Central America		X	X	X		shade

APPENDIX I. Continued.

Species	Ecozone HT—Humid Tropics ASA—Arid/ Semiarid TH—Tropical Highlands	Countries (Main countries or regions of use)	End uses: Fuelwood	Livestock fodder	Green manure	Poles and posts	Erosion control	Other
Gliricidia sepium	HT (ASA)	West Africa, Central America, Philippines, Sri Lanka	X	X	X	X	X	
Inga vera	TH	Central America, Caribbean	X		X	X	X	shade
Intsia bijuga	HT	Southeast Asia, India	X					timber
Leucaena diversifolia	TH (HT, ASA)	India, Central America	X	X	X	X	X	
L. leucocephala	HT (ASA)	Pantropical	X	X	X	X	X	
Mimosa scabrella	HT	South America	X			X	X	shade
Parkia javanica	HT	Pantropical	X					timber
P. speciosa	HT	Thailand, Malaysia	X					food
Parkinsonia aculeata	ASA	Africa, India, Central America	X	X		X	X	
Pithecellobium dulce	ASA	India, Africa	X	X		X	X	
Prosopis alba	ASA	South America	X	X		X	X	
P. chilensis	ASA	South America	X	X		X	X	
P. cineraria	ASA	India	X	X	X	X	X	
P. juliflora/pallida	ASA	India, Africa	X	X		X	X	
P. tamarugo	ASA	Chile	X	X		X		
Robinia pseudoacacia	TH	South and Central Europe, India, Korea	X	X	X	X	X	
Samanea saman	ASA (HT)	Africa, South and Central America	X	X		X		
Sesbania grandiflora	ASA (HT)	India	X	X	X		X	
S. sesban	ASA (HT)	India, Africa	X	X	X			food

[1]Also known as *Pongamia pinnata*.

APPENDIX II. Woody legume weed species.

Species	Countries	Major pest	Recorded weed	Source
Acacia albortieata	Bolivia		x	Holm et al. (1979)
A. aneura	Australia		x	Hodgkinson (1983)
A. armata	Australia		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
A. argyrodendron	Australia		x	Kleinschmidt & Johnson (1977)
A. aroma	Argentina		x	Holm et al. (1979)
A. atramentaria	Argentina		x	Holm et al. (1979)
A. baileyana	South Africa		x	Wells et al. (1986a)
A. berlandieri	U.S.A.		x	Holm et al. (1979)

APPENDIX II. Continued.

Species	Countries	Major pest	Recorded weed	Source
A. bidwillii	Australia		x	Kleinschmidt & Johnson (1977)
A. bonariensis	Argentina		x	Holm et al. (1979)
A. brevispica	Kenya		x	Ivens (1967)
A. cambagei	Australia		x	Kleinschmidt & Johnson (1977)
A. catechu	Kenya		x	Ivens (1967)
A. collinsii	Mexico and Central America		x	Janzen (1974)
A. constricta	U.S.A.		x	Holm et al. (1979)
A. cornigera	Mexico and Central America		x	Janzen (1974)
A. cyanophylla	South Africa		x	Wells et al. (1986a)
(syn. *A. saligna*)	Australia		x	Holm et al. (1979)
A. cyclops	South Africa	x		Wells & Stirton (1982)
A. dealbata	Australia	x		Holm et al. (1979)
	South Africa	x		Wells et al. (1986)
	New Zealand		x	Holm et al. (1979)
A. decurrens	U.S.A.		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
	South Africa		x	Wells & Stirton (1982)
A. drepanolobium	Kenya		x	Ivens (1967)
A. erubescens	Zimbabwe		x	Holm et al. (1979)
A. farnesiana	Australia	x		Moore (1971)
	South Africa		x	Wells et al. (1986a)
	Mexico		x	Holm et al. (1979)
	Fiji		x	Holm et al. (1979)
	Paraguay	x		Holm et al. (1979)
	U.S.A.	x		Scifres et al. (1982)
A. fimbriata	South Africa		x	Wells et al. (1986a)
A. flavescens	Australia		x	Kleinschmidt & Johnson (1977)
A. georginae	Australia		x	Moore (1971)
A. gerrardii	Zimbabwe		x	Holm et al. (1979)
	Kenya		x	Ivens (1967)
A. glaucescens	Australia		x	Holm et al. (1979)
A. glomerosa	Venezuela		x	Holm et al. (1979)
A. greggii	U.S.A.		x	Holm et al. (1979)
A. harpophylla	Australia		x	Kleinschmidt & Johnson (1977)
A. heblecadoides	Tanzania		x	Holm et al. (1979)
	Kenya		x	Holm et al. (1979)
A. heteracantha	South Africa		x	Holm et al. (1979)
	Zimbabwe		x	Holm et al. (1979)
A. hindsii	Mexico and Central America		x	Janzen (1974)
A. hockii	Uganda	x		Harrington (in press)
	Kenya	x		Ivens (1967)
A. implexa	South Africa		x	Wells et al. (1986a)
A. karoo	South Africa		x	Wells & Stirton (1982)
	Zimbabwe		x	Holm et al. (1979)
A. lahai	Kenya		x	Ivens (1967)
A. longifolia	South Africa	x		Wells et al. (1986a)
	New Zealand		x	Holm et al. (1979)
A. mangium	Australia		x	Holm et al. (1979)
A. mearnsii	South Africa	x		Wells et al. (1986a)
A. melanoxylon	South Africa	x		Wells et al. (1986a)

APPENDIX II. Continued.

Species	Countries	Major pest	Recorded weed	Source
	Australia		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
A. mellifera (syn. *A. detinens*)	South Africa		x	Wells & Stirton (1982)
A. mollissima (syn. *A. decurrens*)	Australia		x	Holm et al. (1979)
A. nilotica	South Africa		x	Wells & Stirton (1982)
	Australia	x		Kleinschmidt & Johnson (1977)
	East Africa		x	Ivens (1967)
A. pennata	Kenya		x	Holm et al. (1979)
	India		x	Holm et al. (1979)
A. pennatula	Mexico		x	Chazaro Basanez (1977)
A. podalyriifolia	South Africa		x	Wells et al. (1986a)
A. pycantha	South Africa		x	Wells et al. (1986a)
A. rehmanniana	Zimbabwe		x	Holm et al. (1979)
A. retinoides	Australia		x	Holm et al. (1979)
A. rigidula	Mexico and U.S.A.		x	Holm et al. (1979)
A. saligna (syn. *A. cyanophylla*)	South Africa	x		Wells & Stirton (1982)
A. seyal	Kenya		x	Holm et al. (1979)
	Angola		x	Holm et al. (1979)
A. stuhlmannii	Tanzania		x	Holm et al. (1979)
A. subalata	Zimbabwe		x	Holm et al. (1979)
	Kenya		x	Holm et al. (1979)
A. terminalis	South Africa		x	Wells et al. (1986a)
A. tortuosa	Venezuela		x	Holm et al. (1979)
A. zanzibarica	Kenya		x	Ivens (1967)
Albizia amara	Zimbabwe		x	Holm et al. (1979)
A. chinensis	Indonesia		x	Holm et al. (1979)
A. falcataria	Indonesia		x	Holm et al. (1979)
	U.S.A.		x	Holm et al. (1979)
A. lebbek	Fiji		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
A. lophantha	Australia		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
	South Africa	x		Wells & Stirton (1982)
A. procera	South Africa		x	Wells et al. (1986a)
Alhagi camelorum	Australia		x	Parsons (1973)
	U.S.A.		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
Andira humilis	Brazil		x	Holm et al. (1979)
A. inermis	Jamaica		x	Holm et al. (1979)
Bauhinia cuyabensis	Brazil		x	Holm et al. (1979)
B. variegata	South Africa		x	Wells et al. (1986a)
Brachystegia spiciformis	Zimbabwe		x	Holm et al. (1979)
Caesalpinia bonduc	U.S.A.		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
	Jamaica		x	Holm et al. (1979)
C. coriaria	Jamaica		x	Holm et al. (1979)
C. crista	U.S.A. (Hawaii)		x	Holm et al. (1979)

APPENDIX II. Continued.

Species	Countries	Major pest	Recorded weed	Source
C. decapetala	Kenya		x	Ivens (1967)
	Zimbabwe		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
C. pulcherrima	South Africa		x	Wells et al. (1986a)
	Central America		x	Hughes (unpublished)
C. sepiaria	Australia		x	Diatloff (pers. comm.)
Cajanus cajan	South Africa		x	Wells et al. (1986a)
	Indonesia		x	Holm et al. (1979)
	Jamaica		x	Holm et al. (1979)
	Philippines		x	Holm et al. (1979)
Calicotome spinosa	Australia		x	Lamp & Collet (1979)
Cassia alata	Papua New Guinea		x	Henty & Pritchard (1973)
C. bicapsularis	South Africa		x	Wells et al. (1986a)
C. coliteoides	South Africa		x	Wells et al. (1986a)
C. corymbosa	South Africa		x	Wells et al. (1986a)
C. didymobotrya	South Africa		x	Neser (pers. comm.)
C. eremophila	Australia		x	Hodgkinson (1983)
C. fistula	Jamaica		x	Holm et al. (1979)
C. floribunda	South Africa		x	Wells et al. (1986a)
C. hirsuta	South Africa		x	Wells et al. (1986a)
C. nemophila	Australia		x	Hodgkinson (1983)
C. obtusifolia	Vanuatu,		x	Terry (1982)
	Solomons		x	Terry (1982)
	West Africa		x	Terry (1983)
	Fiji		x	Terry (1982)
C. occidentalis	West Africa		x	Ivens (1967)
C. pallida	Mexico and			Hughes (unpublished)
(syn. *C. biflora*)	Central America		x	
C. rotundifolia	West Africa		x	Hepper (pers. comm.)
C. siamea	South Africa		x	Wells et al. (1986a)
C. surattensis	South Africa		x	Wells et al. (1986a)
C. tomentosa	South Africa	x	x	Wells et al. (1986a)
Ceratonia siliqua	South Africa		x	Wells et al. (1986a)
Cercidium floridum	U.S.A.		x	Holm et al. (1979)
C. macrum	U.S.A.		x	Holm et al. (1979)
C. microphyllum	U.S.A.		x	Holm et al. (1979)
Cercis canadensis	U.S.A.		x	Holm et al. (1979)
C. occidentalis	U.S.A.		x	Holm et al. (1979)
Chamaecytisus proliferus	Australia		x	Lamp & Collet (1979)
Colophospermum mopane	Zimbabwe		x	Holm et al. (1979)
	South Africa		x	Wells & Stirton (1982)
Crotalaria agatiflora	South Africa		x	Wells et al. (1986a)
Cytisus scoparius	South Africa		x	Wells et al. (1986a)
Desmanthus virgatus	South Africa		x	Ivens (1967)
Dicrostachys cinerea	Cuba		x	Liogier (1969)
	East Africa		x	Ivens (1967)
Enterolobium cyclocarpum	Venezuela		x	Holm et al. (1979)
Erythrina abyssinica	South Africa		x	Wells et al. (1986a)

APPENDIX II. Continued.

Species	Countries	Major pest	Recorded weed	Source
E. crista	South Africa		x	Wells et al. (1986a)
Genista linifolia	Australia		x	Lamp & Collet (1979)
G. monspessularia	Australia		x	Lamp & Collet (1979)
Geoffraea decorticans	Argentina		x	Holm et al. (1979)
Gleditsia triacanthos	U.S.A.		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
Gliricidia sepium	Jamaica		x	Holm et al. (1979)
Laburnum anagyroides	Turkey		x	Holm et al. (1979)
Leucaena leucocephala	Ghana		x	Holm et al. (1979)
	U.S.A. (Hawaii)		x	Holm et al. (1979)
	Indonesia		x	Holm et al. (1979)
	Trinidad		x	Holm et al. (1979)
	Nigeria		x	Holm et al. (1979)
	Vanuatu		x	Cock (1982)
	South Africa		x	Wells et al. (1986a)
Lupinus arboreus	Chile		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
Mimosa invisa	Pantropical	x		Holm et al. (1979)
M. pigra	Australia	x		Miller et al. (1981)
	Thailand	x		Napompeth (1982)
	South Africa		x	Wells et al. (1986a)
	Pantropical		x	Holm et al. (1979)
M. pudica	Pantropical	x		Holm et al. (1979)
M. somnians	Colombia		x	Holm et al. (1979)
M. tenuiflora	Mexico and Central America	x		Hughes & Styles (1984)
Parkinsonia aculeata	Australia	x		Miller & Pickering (1980)
	South Africa		x	Wells et al. (1986a)
Pithecellobium dulce	U.S.A. (Hawaii)		x	Holm et al. (1979)
	India		x	Holm et al. (1979)
Prosopis campestris	Paraguay		x	Holm et al. (1979)
P. chilensis	U.S.A. (Hawaii)		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
P. farcta	Israel		x	Holm et al. (1979)
P. glandulosa	U.S.A.	x		Fisher (1977)
	Mexico		x	Holm et al. (1979)
	South Africa	x		Wells et al. (1986a)
	Australia	x		Moore (1971)
P. humilis	Argentina		x	Holm et al. (1979)
P. juliflora	Mexico		x	Holm et al. (1979)
	Australia		x	Holm et al. (1979)
	India		x	Holm et al. (1979)
	Venezuela		x	Holm et al. (1979)
	Colombia		x	Holm et al. (1979)
P. pubescens	South Africa		x	Wells et al. (1986a)
P. ruscifolia	Paraguay	x		D'Antoni & Solbrig (1977)
	Argentina	x		D'Antoni & Solbrig (1977)
P. velutina	U.S.A.	x		Glendening & Paulsen (1955)
	Australia		x	Holm et al. (1979)
	South Africa	x		Wells et al. (1986a)

APPENDIX II. Continued.

Species	Countries	Major pest	Recorded weed	Source
Robinia pseudoacacia	New Zealand		x	Holm et al. (1979)
	U.S.A.		x	Holm et al. (1979)
	South Africa		x	Wells et al. (1986a)
	Australia		x	Holm et al. (1979)
	Israel		x	Holm et al. (1979)
	Europe		x	Holm et al. (1979)
Sarothamnus scoparius	U.K.		x	Roberts (1983)
	Australia		x	Lamp & Collet (1979)
Sesbania bispinosa	Indonesia		x	Holm et al. (1979)
S. grandiflora	Indonesia		x	Holm et al. (1979)
S. punicea	South Africa	x		Wells & Stirton (1982)
S. sesban	Indonesia		x	Holm et al. (1979)
	Philippines		x	Holm et al. (1979)
	Thailand		x	Holm et al. (1979)
	Angola		x	Holm et al. (1979)
Spartium junceum	South Africa		x	Wells et al. (1986a)
Tamarindus indica	South Africa		x	Wells et al. (1986a)
Tipuana tipu	South Africa		x	Wells et al. (1986a)
Ulex europaeus	U.S.A. (Hawaii)		x	Holm et al. (1979)
	New Zealand		x	Holm et al. (1979)
	Chile		x	Holm et al. (1979)
	Iran		x	Holm et al. (1979)
	Italy		x	Holm et al. (1979)
	Poland		x	Holm et al. (1979)
	Australia		x	Lamp & Collet (1979)
	U.K.		x	Roberts (1983)
	South Africa		x	Wells et al. (1986a)

BIOLOGICAL IMPLICATIONS OF GENOME EVOLUTION

H. Rees[1] and R. K. J. Narayan[1]

Abstract

Divergence and speciation within the Leguminosae were accompanied by massive changes in genome size, that is, in the amount of chromosomal DNA. The 2C values vary 30–fold, from less than one picogram in *Lotus corniculatus* to 27 picograms in *Vicia faba*. Surveys within genera provide information about the way in which DNA changes are distributed among individual chromosomes within complements, and the qualitative DNA changes that accompany the change in amount. Surveys in *Vicia* and *Lathyrus* show that the distribution of DNA change is not random among the chromosomes of the complement. On the contrary, with increase in nuclear DNA each chromosome, independently of its length, acquires an equal increment. One consequence is that the chromosome complements of species with high DNA amount are more symmetrical than those with low DNA. As for the quality and composition of the DNA, detailed surveys in *Lathyrus* show remarkably rigid patterns of change in composition with increase in DNA amount. For every additional picogram of nonrepetitive DNA there is an addition of 4.2 picograms of repetitive DNA. For every additional picogram of DNA located in euchromatin there is an increment of 1.2 picograms located in heterochromatin. These well-defined patterns of distribution and quality argue for rigid constraints upon the changes incurred, which in turn are suggestive of strong selective pressures imposed upon the evolution of the chromosome complements. Precisely how these pressures are imposed and what they signify in genetical and physiological terms is conjectural. At the cell level, certainly there are a number of predictable changes in form and behavior which correlate with the quantitative variation in nuclear DNA. They range from the rate of cell division and cell expansion during growth to the amount of recombination at meiosis. The correlations are clear enough—the problem is distinguishing between cause and effect.

I. Introduction

From a cytological standpoint, the legumes exhibit many characteristics that are typical of angiosperm orders in general. In particular, their chromosome complements confirm that divergence and speciation were often accompanied by massive quantitative changes in genetic material, namely in nuclear DNA. In part, the DNA variation is attributable to polyploidy; about a third of the species listed in the chromosome atlas compiled by Darlington & Wylie (1955) are polyploids. Aside from polyploidy, there is an astonishing degree of variation in DNA amounts among the diploid species, including very closely related diploids. This variation results from the amplification, and possibly deletion, of DNA base sequences within the chromosomes. It is with the nature, the distribution, and the consequences of such DNA variation that this paper is concerned.

II. The DNA Amounts

It is convenient and customary to express the nuclear DNA amounts for each species as that which is measured in somatic cells at interphase before DNA synthesis, that is, at G_1. The 2C nuclear DNA amounts from three genera of the Leguminosae, namely, *Phaseolus* (2x = 22), *Lathyrus* (2x = 14), and *Vicia* (2x = 10, 12, 14), are shown in Figure 1. Within all three there is extensive variation

[1]Department of Agricultural Botany, The University College of Wales, Penglais, Aberystwyth, Dyfed SY23 2DD, Wales, U.K.

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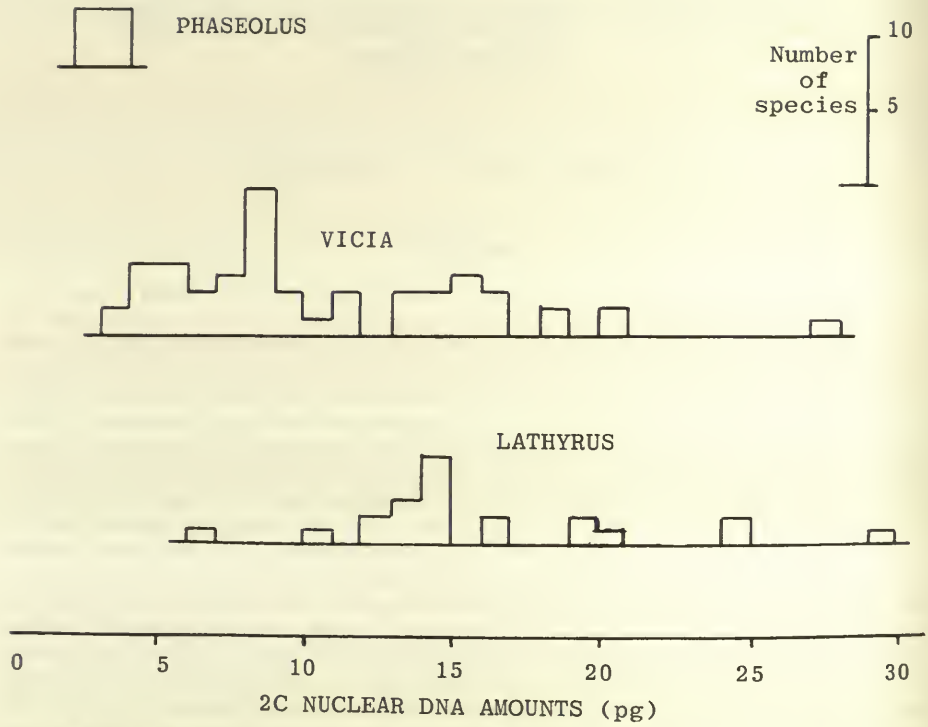


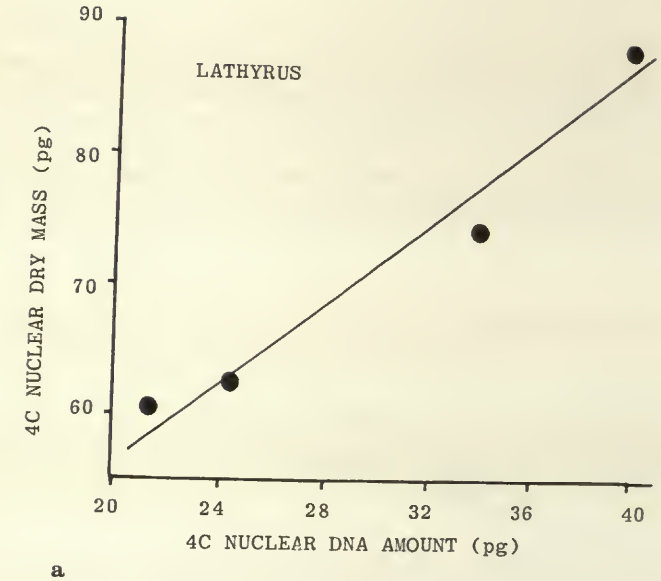
FIGURE 1. The distribution of nuclear DNA amounts in diploid species of *Phaseolus*, *Vicia*, and *Lathyrus*, from Ayonoadu (1974), Raina & Rees (1983), and Narayan & Rees (1976), respectively.

among species. Most striking are the values for *Vicia* species, ranging from 3.85 picograms (pg) in *V. monantha* to 27.07 pg in *V. faba*, a seven-fold difference. The one pair of metacentric chromosomes of *V. faba* contains more DNA than the whole chromosome complement of *V. monantha* (Raina & Rees, 1983). Within the Leguminosae as a whole the range of DNA values among diploids is even greater, from less than 1 pg in *Lotus corniculatus* (Bennett & Smith, 1976) to 27 pg in *V. faba*.

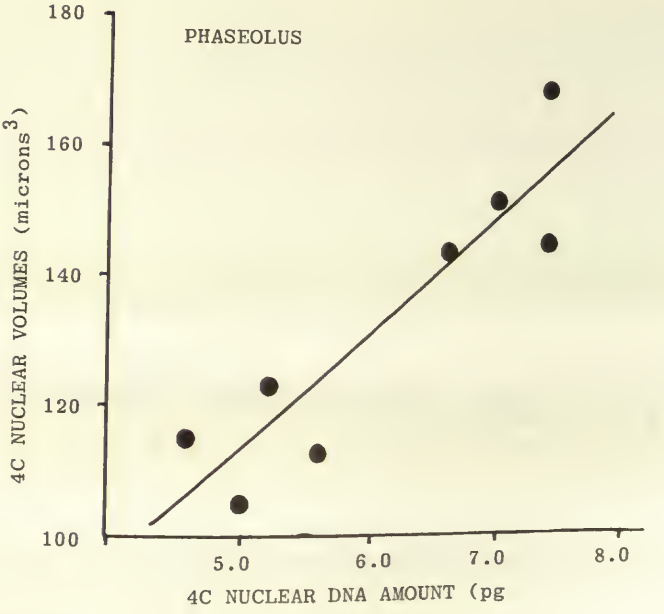
Numerous surveys in a wide range of plant and animal genera show that quantitative change in nuclear DNA is accompanied by correlated changes in cell size and the volume and mass of the nucleus and of its constituent chromosomes (Cavalier-Smith, 1978). In the Leguminosae such correlations have been described in *Lathyrus* (Rees & Hazarika, 1969; Rees & Narayan, 1981), in *Vicia* (Raina & Rees, 1983), and in *Phaseolus* (Ayonoadu, 1974). Examples are given in Figure 2. The morphological consequences at the cellular and multicellular level may be substantial. These, in turn, may affect cell growth and development in ways that we shall consider in a later section.

III. The Distribution of Change Among Chromosomes

Lathyrus. While the differences between species in the total nuclear DNA amount is readily established, the question remains as to how these differences are distributed between the individual chromosomes within the complements. The methods for estimating the nuclear DNA amounts and their constituent components are given by Narayan & Rees (1976). In Figure 3a, the DNA amounts for each chromosome (in rank order, from small to large) in each of four *Lathyrus* species with

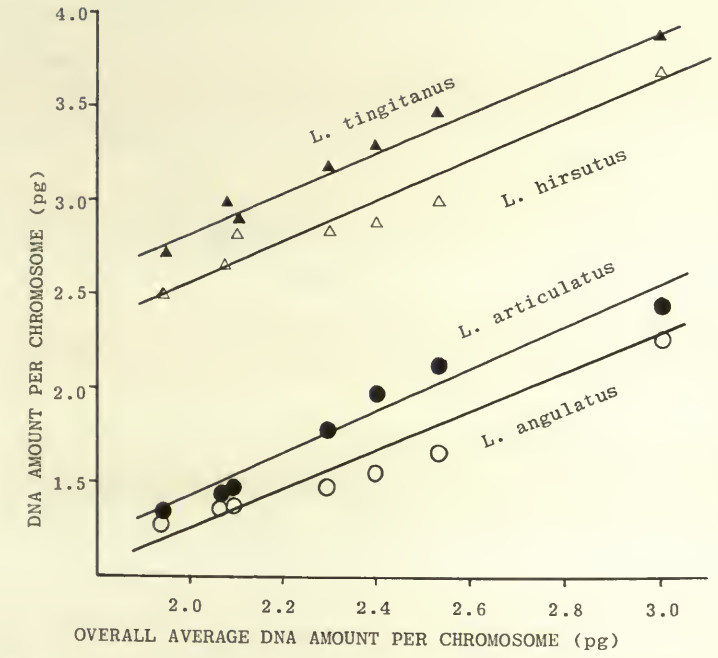


a



b

FIGURE 2. —a. The nuclear dry mass in root meristem cells of four species of *Lathyrus* plotted against the DNA amount in 4C nuclei.—b. The nuclear volume in root meristems of *Phaseolus* species plotted against the DNA amounts in 4C nuclei.



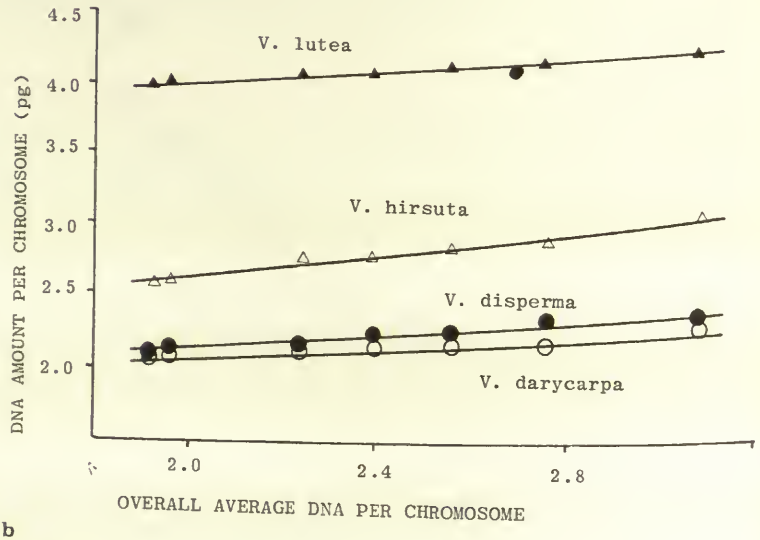


FIGURE 3. The DNA amounts in chromosomes of (a) *Lathyrus* and (b) *Vicia* species in rank order of size plotted against the overall mean DNA amounts of the chromosomes, also in rank order; data from Narayan & Durrant (1983), and Raina & Rees (1983), respectively.

different nuclear DNA amounts is plotted against the mean DNA amount of each of the seven chromosomes (also in rank order, from all four species). The figure shows four closely parallel lines with a positive slope. An analysis of variance confirms a significant joint regression and, as would be expected, a significant difference between the mean DNA amounts of the four species. More importantly, the analysis shows no significant heterogeneity between the slopes of the regression lines, confirming their parallel alignment. It is the parallel nature of the regression lines that tells us how the extra DNA is distributed among the individual chromosomes with increase in total DNA amount. It tells us that each chromosome within the complement, independent of its size, acquires precisely the same DNA increment, namely one-seventh of the total. If, as might have been expected, the increase in DNA were distributed at random within the complement, the DNA increment for each chromosome would be proportional to its length with the smaller chromosomes acquiring less than the larger. Under these circumstances the regression lines in Figure 3a would diverge from left to right rather than maintain their parallel alignment.

It follows from the above that the amplification of DNA base sequences among the chromosomes within complements is not random. On the contrary, it is strictly constrained. One consequence of such a constraint is that with increasing nuclear DNA amount the relative lengths of the chromosomes within a complement become more similar, and thus the complement more "symmetrical." This is illustrated clearly in Figure 4.

Vicia. Figure 3b shows for four *Vicia* species with $2x = 14$ precisely the same pattern with the same constraint upon the distribution of change as in *Lathyrus*. Comparable examples from species in other families of plants have been described by Seal & Rees (1982) and by White & Rees (1986).

EUCHROMATIN VERSUS HETEROCHROMATIN

By scanning interphase nuclei at different optical densities, it is possible to measure separately the amount of DNA located within euchromatin and heterochromatin. Figure 5a shows the DNA amount in each of the two components plotted against the total DNA in four *Lathyrus* species. With increase in the total there is increase in the DNA located both within euchromatin and heterochromatin. The regression lines are linear and the slope for the DNA amounts in heterochromatin is steeper than that for euchromatin. The linearity of the regression lines establishes that the extra DNA located in euchromatin relative to heterochromatin, with increase in the total, is constant within the species. That the slope is steeper for the heterochromatic fraction signifies that its rate of increase is greater than that for the euchromatin. In fact, for each additional picogram of DNA in euchromatin there is an additional 1.2 pg in heterochromatin.

REPETITIVE VERSUS NONREPETITIVE DNA

Figure 5b shows the amount of nonrepetitive and repetitive DNA in *Lathyrus* species with different total nuclear DNA amounts. The regression lines are linear and diverging. For every additional picogram of nonrepetitive DNA there is a consistent increase of 4.2 picograms of repetitive DNA.

IV. Constraints

The results above establish that the distributions of quantitative DNA changes, among the different chromosomes within complements and among different structural and molecular components within the chromosomes, are by no means random. On the contrary, there are constraints upon the distribution of DNA change such that they conform to well-defined and surprisingly consistent

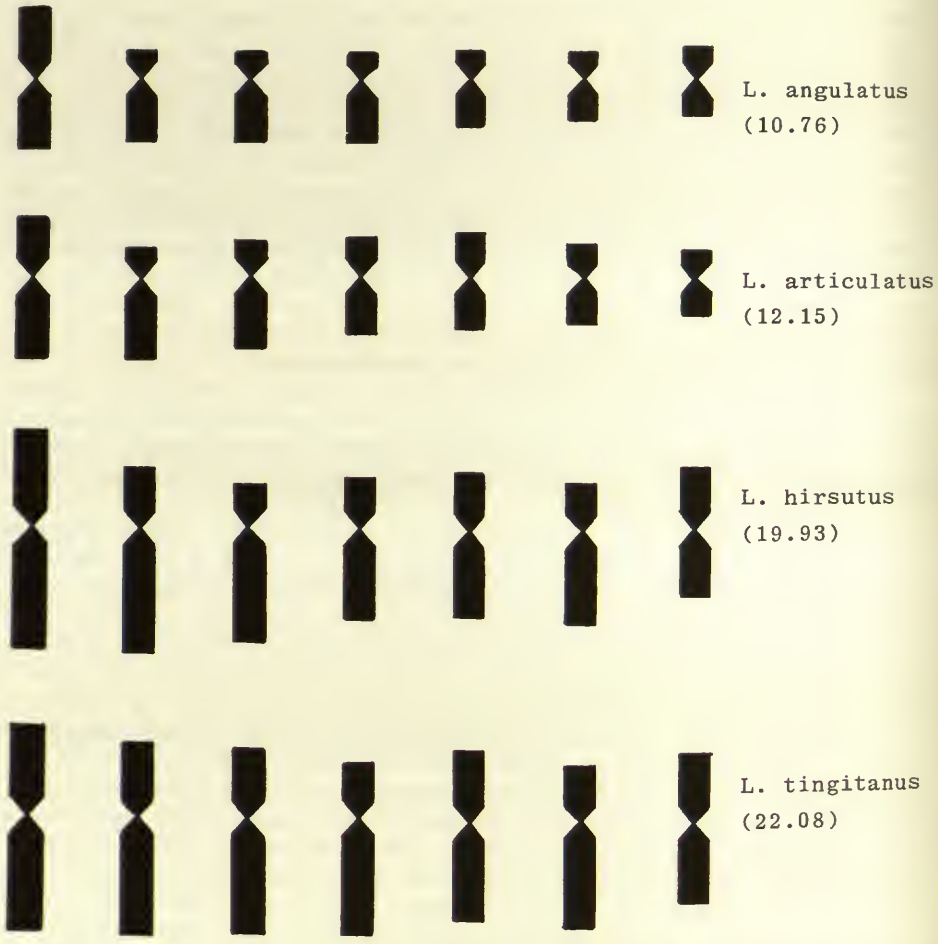


FIGURE 4. The chromosome complements of four species of *Lathyrus* with 2C DNA amounts in brackets.

patterns. We argue that such constraints are construed most readily as consequences of selection and, by implication, of functional and adaptive significance (Rees & Narayan, 1981).

V. REPEATED SEQUENCES

By centrifugation in a CsCl gradient in the presence of actinomycin D, it is possible to isolate particular satellite nuclear DNA fractions of a highly repetitive nature. It is also possible to "hybridize" in situ these satellite DNAs (or their *c*RNA transcripts) to the denatured DNA of the chromosomes from which they derive and, thereby, to determine their location. Figure 6a illustrates the results of the in situ hybridization of one such satellite. It derives from *Lathyrus tingitanus* and is comprised of a sequence of some 450 base pairs repeated about one million times. The tritium labeled *c*RNA transcribed by this satellite, using RNA polymerase, binds to the heterochromatic segments on hybridization in situ to the *L. tingitanus* chromosomes. This is not surprising since it is well known that the heterochromatin is rich in highly repeated sequences of the satellite type.

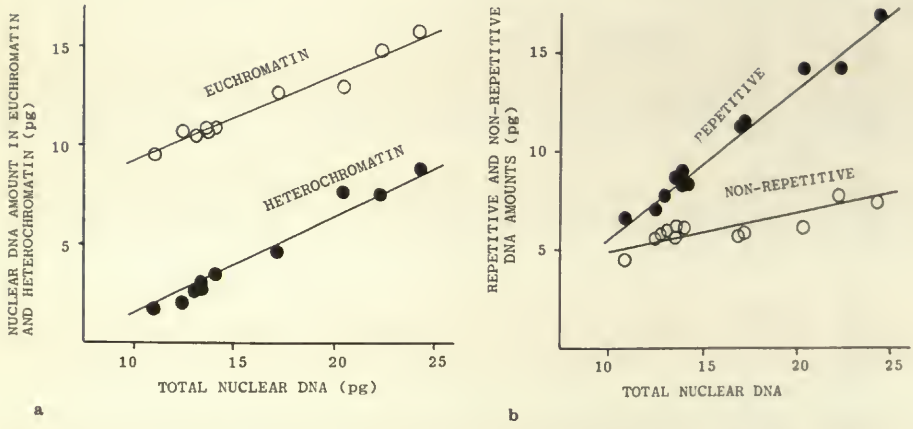


FIGURE 5. DNA amounts located in heterochromatin and euchromatin (a) and in repetitive and nonrepetitive fractions (b) plotted against the total nuclear DNA amount in *Lathyrus* species.

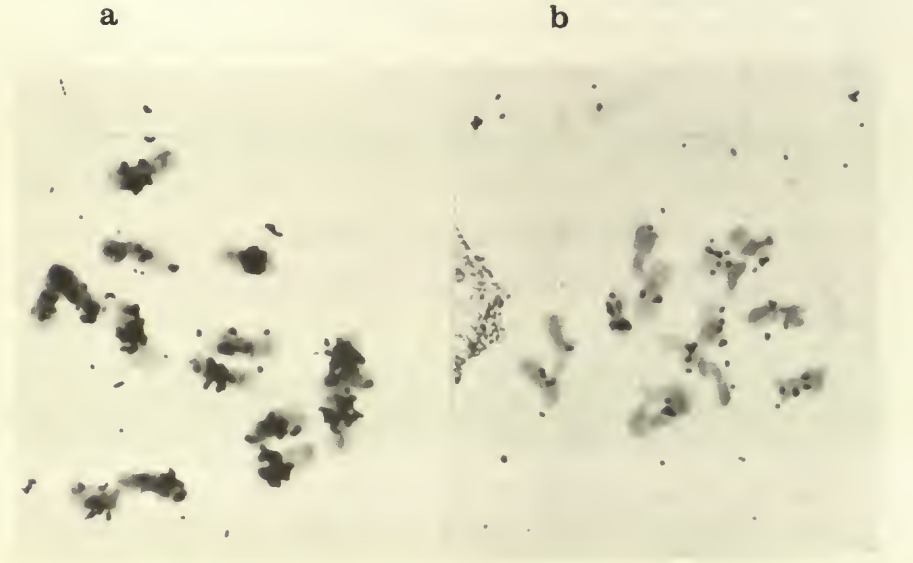


FIGURE 6. Autoradiograph showing in situ hybridization of labeled *c*RNA derived from a satellite DNA of *Lathyrus tingitanus* on to *L. tingitanus* chromosomes (a), and (b) the *c*RNA from the *L. tingitanus* satellite hybridized to the chromosomes of *L. sativus*.

Two questions which now arise are: whether the same repetitive sequence is found in related species; if so, whether the sequence is confined, as in *Lathyrus tingitanus*, to the heterochromatin. In situ hybridization of the labeled *c*RNA transcript from the *L. tingitanus* satellite to the chromosomes of *L. hirsutus*, *L. ochrus*, and *L. sativus* show that repeats of this sequence are rare in these other species (see Fig. 6b). Moreover, its location is no longer restricted to the heterochromatin. Clearly, the sequence is not highly conserved within the genus. There is also evidence for its dispersion during speciation. Mechanisms to account for the modification and dispersion of such

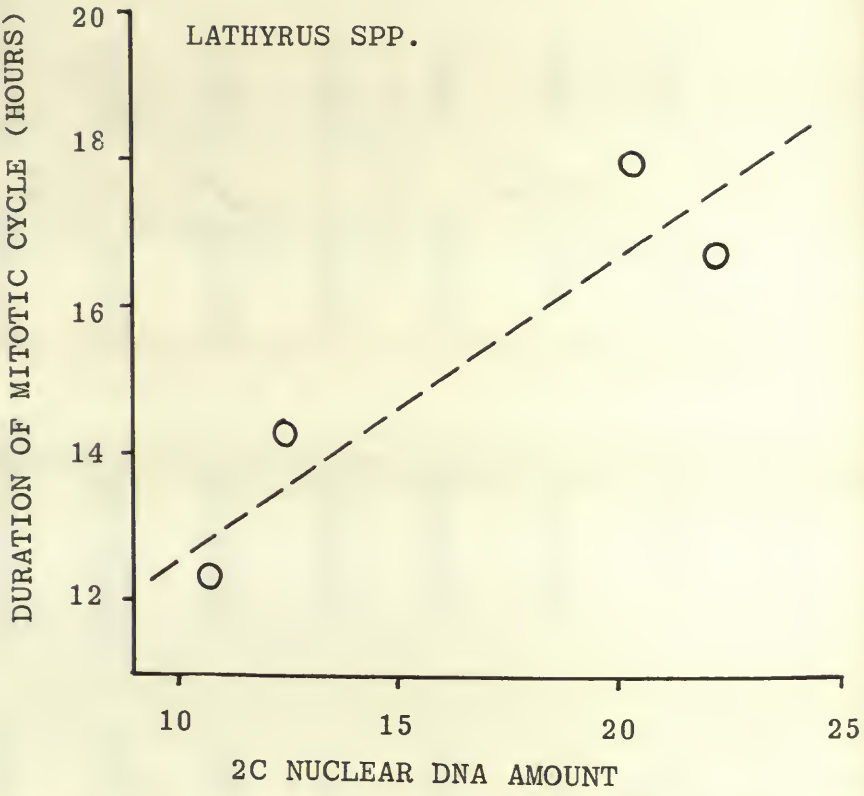


FIGURE 7. The duration of mitotic cycle in root meristems plotted against the nuclear DNA amounts in *Lathyrus* species.

repeated sequences have been discussed elsewhere in detail (e.g., Flavell, 1982), and need not be considered here. What needs to be emphasized is that the molecular composition of the repeated sequences in different *Lathyrus* species varies substantially. Therefore, if we consider that the repeated sequences within the *Lathyrus* chromosomes are of functional importance, such functions would appear to be independent of their base sequence composition.

VI. Cell Division

We have described the phenotypic changes at the cellular and intracellular levels which are associated with change in nuclear DNA amounts. At the cell level two other consequences of the nuclear DNA variation demand consideration. They relate to function rather than form, to behavior rather than morphology.

THE MITOTIC CYCLE

Figure 7 shows the duration of the mitotic cycle in root meristems of *Lathyrus* species with different nuclear DNA amounts. As the DNA amount increases so does the duration of the cycle. At 20°C it increases by about 15 minutes per picogram (cf. Van t'Hof, 1965; Evans et al., 1972).

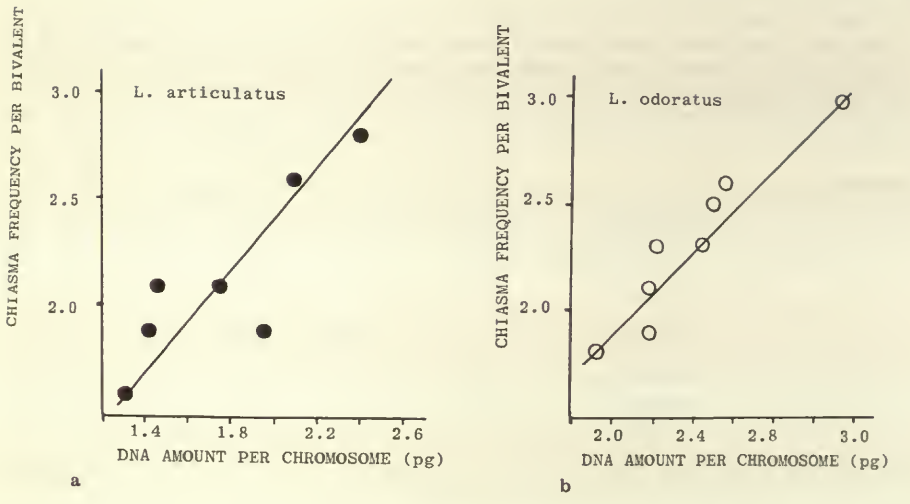


FIGURE 8. The chiasma frequency per bivalent plotted against the DNA amount per chromosome in (a) *Lathyrus articulatus* and (b) *L. odoratus*. Solid lines represent expected values assuming the chiasma frequency is directly proportional to the DNA amount.

RECOMBINATION AT MEIOSIS

Within Complements

The mean chiasma frequencies, which reflect the amount of recombination, and the DNA amounts for individual bivalents of the complements of plants from two species of *Lathyrus* (*L. articulatus* and *L. odoratus*) are illustrated in Figure 8 and Table 1. Both have relatively low (12.15 pg) and high (16.96 pg) nuclear DNA amounts respectively. In both cases the bivalent chiasma frequency is directly proportional to its DNA content, that is, as the DNA amount within a bivalent doubles so does its chiasma frequency. It follows that the amount of recombination per unit of DNA within each chromosome within a complement is constant. This constancy is reflected in Figure 9, where the distributions of chiasma frequencies per picogram of DNA in the bivalents of both species are plotted against the DNA amount per chromosome. The solid lines in the figure represent the values expected on the basis of a constant chiasma frequency per picogram in each species. The observed values correspond closely with those expected to give expression lines with a slope of 0.

Between Complements

In light of the observations within complements, which show a direct increase in the amount of recombination with increase in DNA amount, it would be expected that the chiasma frequency of different species increases with increasing amounts of nuclear DNA. Figure 9 indicates, however, that this is not so. The chiasma frequency in bivalents with similar nuclear DNA amounts is higher in *Lathyrus articulatus*, which has the low nuclear DNA content, than in *L. odoratus* with the higher nuclear DNA content. In other words, it appears that the amount of recombination per picogram of DNA decreases with an increase in genome size, that is, with increase in the total nuclear DNA. Figure 10 confirms this conclusion. The figure shows the chiasma frequency per picogram of DNA in 12 *Lathyrus* species plotted against the nuclear DNA amount (the genome size). There is a regular

TABLE 1. The mean chiasma frequency and the DNA content (pg per chromosome) of bivalents in pollen mother cells of two *Lathyrus* species. Expected chiasma frequencies assume that the chiasma frequency per pollen mother cell is distributed among bivalents in direct proportion to their DNA content. DNA measurements and chiasma frequencies are means from 20 cells in each case. Data from Fiona McIntyre.

Species		Bivalent						
		1	2	3	4	5	6	7
Lathyrus articulatus	DNA amount	2.42	2.10	1.96	1.76	1.46	1.42	1.32
	Chiasma frequency	2.8	2.5	2.4	2.1	1.8	1.7	1.6
	(expected)	2.8	2.6	1.9	2.1	2.1	1.9	1.6
Lathyrus odoratus	DNA amount	3.15	2.56	2.50	2.43	2.22	2.19	1.91
	Chiasma frequency	3.0	2.6	2.5	2.3	2.3	1.9	1.8
	(expected)	3.0	2.5	2.4	2.3	2.1	2.1	1.8

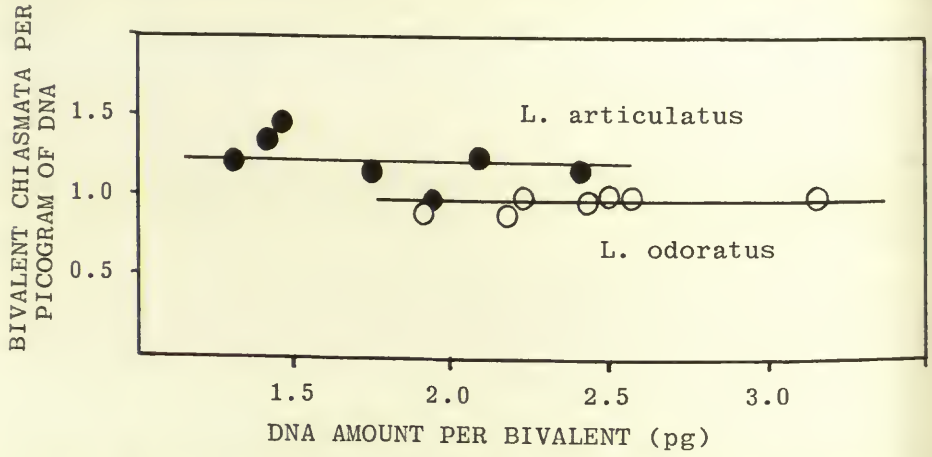


FIGURE 9. The chiasma frequency per picogram of DNA plotted against the DNA amount per chromosome in *Lathyrus articulatus* and *L. odoratus*. Solid lines represent expected values assuming that the chiasma frequency is directly proportional to the DNA amount per chromosome.

pattern of decreasing recombination per unit of DNA with increase in genome size (cf. Rees & Durrant, 1986).

In retrospect, the pattern described herein might have been anticipated on structural grounds. As the total nuclear DNA increases so does the proportion of heterochromatin relative to euchromatin. It is well known that there is no crossing-over within heterochromatin and that heterochromatin in some instances interferes with crossing-over in its vicinity (John & King, 1980). As the total nuclear DNA increases, therefore, so does the proportion of DNA with the capacity for crossing-over and chiasma formation decrease. In genetic terms, the consequence of the constraint upon crossing-over with increasing genome size must inevitably impose a limitation upon the release of genetic variability exposed to selection within the gametes and their offspring.

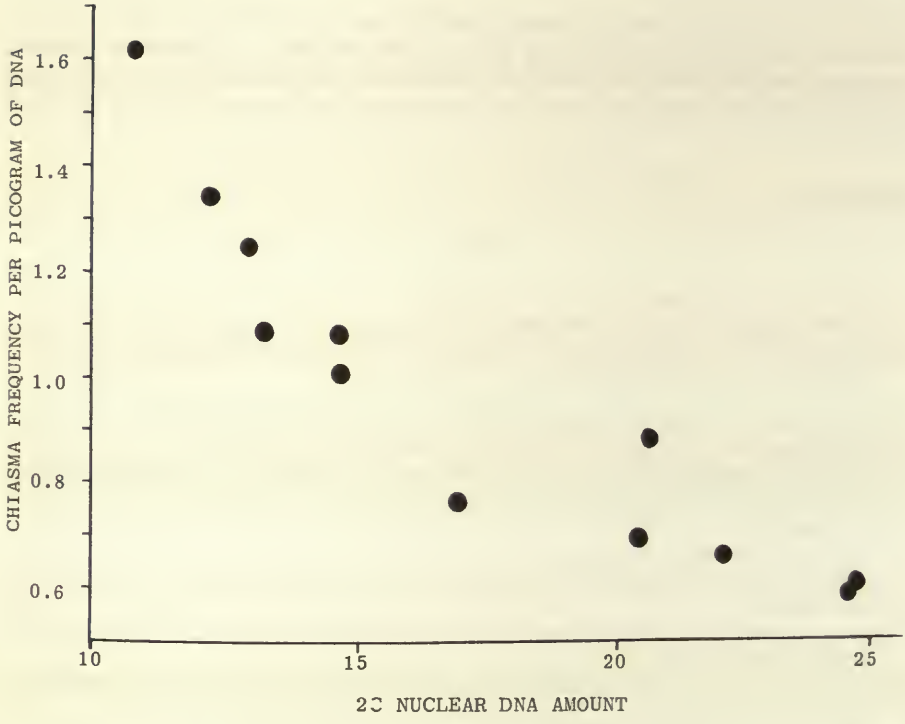


FIGURE 10. The average chiasma frequency per pollen mother cell per picogram of DNA plotted against the 2C nuclear DNA amounts in *Lathyrus* species. Data from Rees & Durrant (1986).

VII. DISCUSSION

The information presented confirms that divergence and speciation within the Leguminosae are often accompanied by massive changes in nuclear DNA amount. These changes, in turn, are accompanied by correlated changes in cell and nuclear volumes, chromosome size, composition of the chromosomal DNA, duration of mitotic cycles, and the capacity for chiasma formation at meiosis. Change in cell volumes and in the duration of mitotic cycles must clearly affect metabolism and growth at cell level (see Cavalier-Smith, 1978). However, when we turn to the level of the whole plant phenotype, there is surprisingly no obvious correlation between the growth and habit of species, their distribution, and nuclear DNA content. Between form and function and the nuclear DNA amount there is no indication of cause and effect. In other words, the so-called *c*-value paradox persists and still applies to the eukaryotes in general.

Much of the extra DNA which results from amplification during evolution may be of a "selfish" and redundant nature (e.g., Doolittle & Sapienza, 1980). At the same time, there are strong indications that the variation in nuclear DNA amount is subject, at least in part, to selection and thereby of adaptive significance. The main justification for this view stems from the constraints upon the distribution of the DNA changes. It will be recalled in particular that the distribution of the DNA change between and within the chromosomes is not random but subject to rigid constraints that generate well-defined patterns of evolution among the chromosome complements of different

genera. It is tempting to argue, for example, that the relative sizes and shapes of chromosomes which are determined by such constraints are themselves of adaptive and functional importance (Bennett, 1983; Rees, 1983). By what means these and the other correlated patterns of change achieve their effects is still a matter more for conjecture than affirmation.

References

Ayonoadu, U. W. U. 1974. Nuclear DNA variation in *Phaseolus*. Chromosoma 48: 41–49.

Bennett, M. D. 1983. The genome, the natural karyotype, and biosystematics. Pp. 41–66 *in* W. F. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

_____ & J. B. Smith. 1976. Nuclear DNA amounts in angiosperms. Phil. Trans. Roy. Soc., B, 274: 227–274.

Cavalier-Smith, T. 1978. Nuclear volume by nucleoskeletal DNA, selection for cell volume and cell growth rate and the solution of the c-value paradox. J. Cell Sci. 24: 247–278.

Darlington, C. D. & A. P. Wylie. 1955. Chromosome Atlas of Flowering Plants. Allen & Unwin, London.

Doolittle, W. F. & C. Sapienza. 1980. Selfish genes, the phenotype paradigm and genome evolution. Nature 284: 601–603.

Evans, G. M., H. Rees, C. L. Snell, & S. Sun. 1972. The relationship between nuclear DNA amount and the duration of the mitotic cycle. Pp. 24–31 *in* K. Lewis & C. D. Darlington (editors), Chromosomes Today 3. Longman, London.

Flavell, R. B. 1982. Sequence amplification, deletion and rearrangement: major sources of variation during species divergence. Pp. 301–323 *in* G. A. Dover & R. B. Flavell (editors), Genome Evolution. Academic Press, London.

John, B. & M. King. 1980. Heterochromatin variation in *Cryptobothrus chrysophorus*. III. Synthetic hybrids. Chromosoma 78: 165–186.

Narayan, R. K. J. & A. Durrant. 1983. DNA distribution in chromosomes of *Lathyrus* species. Genetica 61: 47–53.

_____ & H. Rees. 1976. Nuclear DNA variation in *Lathyrus*. Chromosoma 54: 141–154.

Raina, S. N. & H. Rees. 1983. DNA variation between and within complements of *Vicia* species. Heredity 51: 335–346.

Rees, H. 1983. Nuclear DNA variation and the homology of chromosomes. Pp. 87–96 *in* W. F. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

_____ & A. Durrant. 1986. Recombination and genome size. Theoretical and Applied Genetics 73: 72–76.

_____ & M. H. Hazarika. 1969. Pp. 158–165 *in* C. D. Darlington & K. Lewis (editors), Chromosomes Today, Vol. 2. Oliver & Boyd, Edinburgh.

_____ & R. K. J. Narayan. 1981. Chromosomal DNA in higher plants. Proc. Roy. Soc., B, 292: 569–578.

Seal, A. G. & H. Rees. 1982. The distribution of quantitative DNA changes associated with the evolution of diploid *Festuceae*. Heredity 49: 179–190.

Van't Hof, J. 1965. Relationships between mitotic cycle duration, *S* period duration and the average rate of DNA synthesis in root meristems of several plants. Exptl. Cell Res. 39: 48–58.

White, J. A. & H. Rees. 1986. Chromosome weights and measures in *Petunia*. Heredity 58: 139–143.

THE LEGUME–*RHIZOBIUM* EQUATION: A COEVOLUTION OF TWO GENOMES

Desh Pal S. Verma[1,2] and John Stanley[1]

ABSTRACT:

Legume plants evolved as a result of symbiosis with *Rhizobium*, allowing them to grow in nitrogen-deficient soil and produce protein-rich seeds. Some present-day legumes that do not exhibit active association with nitrogen-fixing bacteria appear to have lost this ability due to change in habitat (i.e., growth in nitrogen-rich soil). The early interaction between the bacteria and plant may have been pathogenic or saprophytic, followed by development of host-specificity. A variety of microorganisms classified as "*Rhizobium*" harbor nitrogen-fixation genes and have coevolved with the host plant to optimize symbiotic interactions. The host plant recruited some of the existing genes into specialized functions of the root nodule to form a new subcellular compartment housing the bacteria and to support the symbiotic state. Thus a nitrogen-fixing symbiotic bacteria acts like an "organelle" inside the infected cell of the root nodule and is entirely dependent on the host. The host plant provides all the necessary energy and develops a suitable environment for the inhabiting bacteria to commence fixing nitrogen. The bacteria has evolved to make use of the existing carbon and nitrogen assimilatory pathways in their respective hosts. Thus coevolution in plant and bacteria has given rise to present-day legumes.

I. INTRODUCTION. LEGUMES AND RHIZOBIA IN EVOLUTION

The Leguminosae constitute the third-largest family of higher plants, with 650 genera and 18,000 species (Allen & Allen, 1981). The Mimosoideae (2,500 species) and Papilionoideae (Crepet & Taylor, 1985) are generally more specialized than Caesalpinioideae. The success of mimosoid and papilionoid legumes is due apparently to the evolution of their ability to harvest the ecological resource of fixed nitrogen using procaryotic nitrogen-fixing microorganisms (Sprent, 1981). A corresponding array of soil bacteria classified in the eubacterial genus *Rhizobium* have evolved the ability to enter endosymbiosis with legumes. Slow- and fast-growing rhizobia differ radically in DNA hybridization and base ratios, serology, exopolysaccharide composition, carbohydrate metabolism, and intrinsic antibiotic resistance (Jordan, 1982).

Economically important temperate perennial legumes, such as *Trifolium* or *Medicago*, as well as such annuals as *Pisum* and *Vicia* are nodulated by fast-growing rhizobia. Tropical grain legumes, such as *Vigna* and *Glycine max*, are effectively nodulated primarily by bradyrhizobia. Symbiotic potential evidently gave legumes the ability to grow in nitrogen-poor soils, a major factor in their global distribution. With evolutionary advances in a host plant, concomitant changes in microsymbiont bacteria presumably demanded the initial evolution of genes specifying nodulation. Similar to host-pathogen systems, the specificity of interaction between legumes and rhizobia has increased and is reflected in the "cross-inoculation" group. The resulting "species" concept for rhizobia can be misleading in specific instances where the possession of host-specificity genes of common functionality causes rhizobia, highly divergent at the genome level, to be regarded as a single species. An example is *Bradyrhizobium japonicum* (Fig. 1), which really comprises two narrower species (Hollis et al., 1981; Stanley et al., 1985). This is discussed in detail in a later section.

[1]Centre for Plant Molecular Biology, Biology Department, McGill University, 1205 Docteur Penfield Ave., Montreal, Quebec.

[2]Present address: Department of Molecular Genetics and Biotechnology Center, Ohio State University, Columbus, Ohio 43210, U.S.A.

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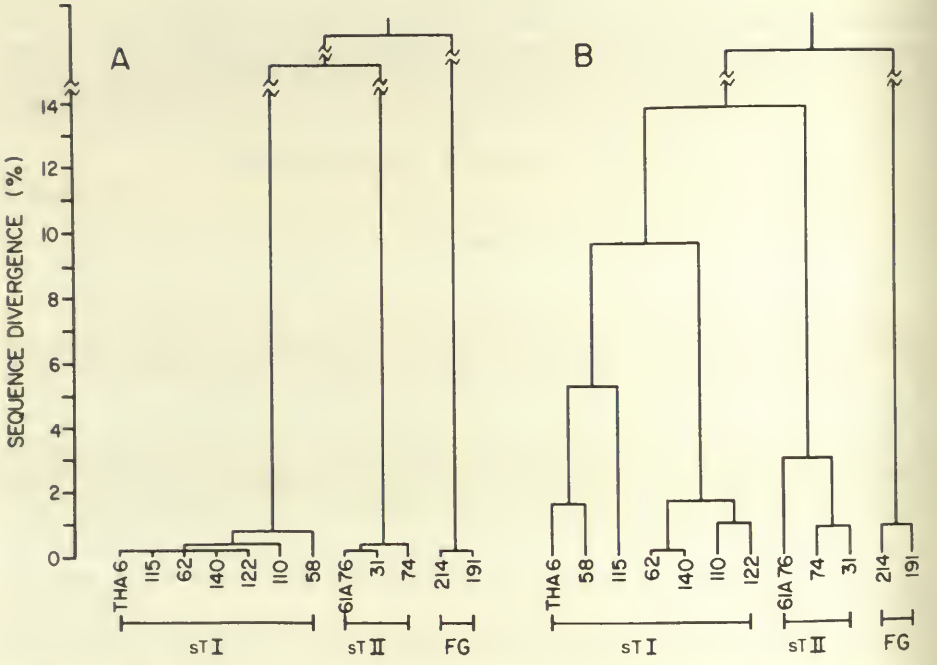

FIGURE 1. Phenograms constructed by the unweighted group pair method to depict the interrelationship among the genomes of various *Bradyrhizobium japonicum* strains isolated at different times and sites in North America and South America. Two fast-growing soybean rhizobia ('FG') from the People's Republic of China were also examined. The vertical axis describes the percent sequence divergence in and around the *nifDH* and *nod* genes (phenogram A) or constitutively expressed genes *recA*, *glnA*, and other cloned sequences (phenogram B) (also see Stanley et al., 1985).

II. The Nature of Symbiotic Genes

Figure 2 shows that the formation of the root nodule involves coordinate expression of essential symbiotic genes in host plant and microsymbiont (Verma & Nadler, 1984; Rolfe & Shine, 1984). In the plant these genes direct the synthesis of nodule-specific host proteins called 'nodulins' (Legocki & Verma, 1980; Verma et al., 1986). Nodulins are involved in structure, maintenance, and overall metabolism of the root nodule. The genes concerned are not expressed in the uninfected legume. Sequence analysis of the DNA of some of these genes suggests that they may have been generated recently in evolution (e.g., nodulin-24, see below) and that several of them evolved from a common ancestor (Jacobs et al., 1986). These genes carry out metabolic and structural functions in the nodule (e.g., tissue-specific carbon-nitrogen assimilation and establishment of subcellular compartment enclosing the bacteria).

The evolution of tissue-specific eucaryotic genes can occur via the duplication of existing genes and the acquisition of specific regulatory elements. The nodule-specific glutamine synthetase of *Phaseolus vulgaris* resembles the root enzyme but appears to possess a specific subunit unique to the nodule enzyme (Lara et al., 1983). Another nodule-specific compartmentalized protein is the tetrameric Uricase II (Bergmann et al., 1983). Its subunit, nodulin-35, is synthesized exclusively in uninfected cells of soybean root nodules and localized in peroxisomes of these cells (Fig. 3). The nodulin-35 gene has been fully characterized at the DNA sequence level (Nguyen et al., 1985).

A very wide variety of soil bacteria from the gram-positive aerobes *(Bacillus)* or anaerobes

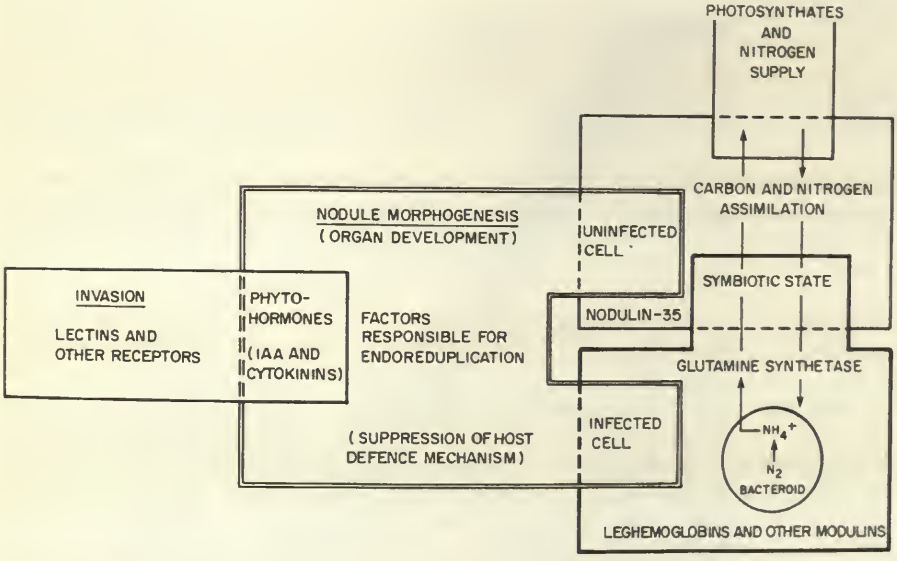

FIGURE 2. Three main phases in the development of the determinate root nodule. Events in these phases are determined by the legume host and *Rhizobium* genomes.

(Clostridium) to gram-negative anaerobes *(Klebsiella)* or aerobes *(Azotobacter)* fix atmospheric nitrogen. The majority of these are free-living diazotrophs. Reflecting the functional constraints of the nitrogenase proteins, the *nif*KDH genes are highly conserved across the genomes of all nitrogen-fixing microorganisms (Ruvkun & Ausubel, 1980). Symbiotic nitrogen fixation can be assumed to result from the evolution of endosymbiotic (Sym) genes by an aerobic diazotroph. The interaction with plants of such associative diazotrophic bacteria as *Azospirillium* (which fixes nitrogen in the rhizosphere of some grasses) and of such pathogenic bacteria as *Pseudomonas savastanoi* (which forms tumors on olive plants) are analogous to associative and pathogenic stages through which a free-living diazotroph could have evolved towards endosymbiosis.

Bacterial symbiotic (*Sym*) genes include constitutively expressed genes, such as those for production of unique exopolysaccharides important in recognition for endosymbiosis (Chakravorty et al., 1982; Finan et al., 1985), and genes whose transcription is symbiosis-specific and depends on plant signals, such as *nod* genes (Mulligan & Long, 1985). Bradyrhizobia can be induced to fix atmospheric nitrogen *ex planta* (McComb et al., 1975; Pagan et al., 1975), under conditions mimicking the microaerophilic environment and appropriate carbon sources of the root nodule. *Nif* genes of fast-growing rhizobia are not generally transcribed *ex planta* under such conditions.

The process by which the nitrogen-fixing endosymbiosis is established may be described in three stages: preinfection (I), infection and nodule formation (II), and nodule function (III). Rhizobia have evolved nodulation *(nod)* genes whose function is required for stages I and II of symbiosis. At least three *nod* clusters occur in the large symbiotic plasmid of fast-growing rhizobia, linked to the *nif* genes. The first of these encodes strongly conserved nodulation genes common to fast-growing rhizobia (Schmidt et al., 1984) and bradyrhizobia (Stanley et al., 1985; Lamb & Hennecke, 1986). The *nod*ABC genes are transcribed coordinately and divergently from *nod*D, and their expression requires *nodD* and the addition of a small-molecular-weight compound of legume root exudate (Mulligan & Long, 1985). Plant-bacterial communication underlies the process of symbiotic nitrogen fixation and characterizes infection and nodule formation (stage II). A second cluster of genes determining host specificity of nodulation maps between *nod*DABC and the nitrogen fixation genes

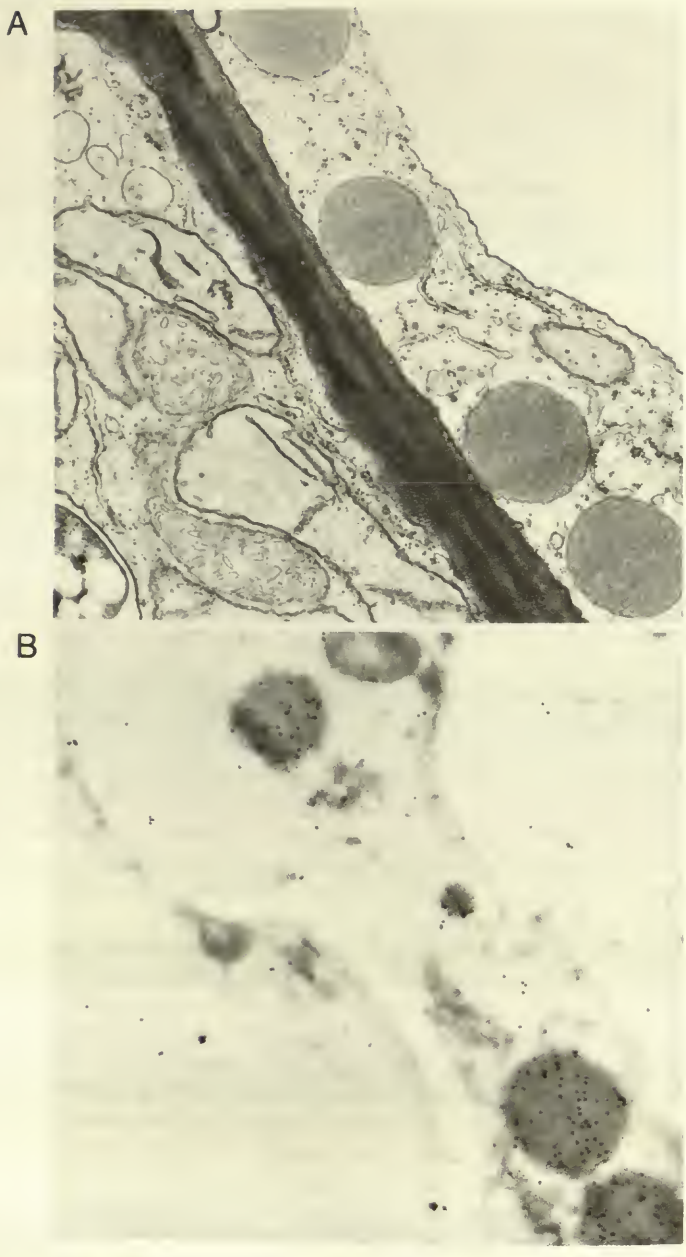

FIGURE 3.—A. Ultrastructural differences between an infected (left) and an uninfected cell (right).—B. Immunocytochemical localization of nodulin-35 in the peroxisomes of an uninfected cell. Note the intensity of Protein-A gold particles identifying nodulin-35 within the peroxisomes (see Nguyen et al., 1985, for details).

on the Sym plasmid of the fast-growing rhizobia, *R. trifolii*, *R. leguminosarum*, and *R. meliloti* (see Djordjevic et al., 1985).

III. Coevolution of Symbiosis

That a coevolution of the eucaryotic plant genome and procaryotic rhizobial bacterium is at work is shown by the reciprocal development of host-specific nodulation between *Pisum sativum* and strains of *R. leguminosarum*. Within the pea species, there is a class of variants originating from the Middle East, e.g., Afghanistan, that is not nodulated by most *R. leguminosarum* strains (Young et al., 1982; Lie, 1978). *R. leguminosarum* strain TOM can nodulate this plant (Winarno & Lie, 1979) as well as all commercial pea varieties. The symbiotic plasmid of strain TOM carries a genetic determinant (dominant in merodiploid bacteria) required for successful infection of Afghanistan peas. Cloning and mutagenesis studies (Hombrecher et al., 1984) indicated that the gene concerned is not a supplemental "host range" gene, but an allelic variant of a *nod* gene, whose possession confers the ability to nodulate Afghanistan as well as commercial peas.

Crosses between a European pea cultivar and primitive wild peas from the Middle East (Lie, 1984) show that at least three plant genes confer resistance to nodulation by European strains of *R. leguminosarum*. The dominant gene Sym-1 controls temperature-sensitive nodulation in primitive peas. Resistance to nodulation with European strains is conferred by the recessive gene sym-2, which is present in the Afghanistan and in six other wild pea cultivars. This gene is linked to the isocitrate dehydrogenase locus of pea chromosome 1, and grafting experiments show that the factor is root-specific (LaRue et al., 1985).

The gene Sym-5 governs a nondiffusable root-associated factor determining resistance to nodulation by all *R. leguminosarum* strains. Sym-4 and Sym-7, which determine nodulation resistance, are nonallelic with Sym-5 (LaRue et al., 1985). These studies in rhizobial and plant mutants indicate that specificity of the symbiosis results from coevolution of the two genomes. Gene centers, the Middle East in the case of *Pisum sativum*, are sources of genetic variants in symbiosis (Lie & Goktan, 1984). The genetic uniformity of European *R. leguminosarum* strains results from domestication and selection of rhizobia from the gene center. Most Middle Eastern strains, adapted to local host plants, form ineffective nodules on modern pea cultivars, although a few form effective nodules. Transfer of these effective rhizobia during the migration of the pea from its gene centers to Europe favored selection of homologous plants by increasing their seed under conditions of limiting soil nitrogen. Conversely, these cultivars favored persistence of their homologous rhizobia. The number of plant and bacterial genotypes in the new environment was low relative to the gene center and mutual specificity evolved over a short period.

A similar situation is found in *Glycine max* with respect to plant genes defined classically, and coevolution with rhizobia or bradyrhizobia (see Devine, 1985). A naturally occurring nodulation mutant of *G. max*, the homozygous recessive pair *rjl rjl*, conditions resistance to nodulation with most *B. japonicum* strains (Williams & Lynch, 1954; Caldwell, 1966) via a root-specific factor. Certain *B. japonicum* strains characterized by a high capsular polysaccharide and high IAA production can overcome the *rjl rjl* block (Hubbell & Elkan, 1966). When *G. max* isolines that are identical except for the *rjl* locus are compared, the wildtype gene favors establishment and persistence of *B. japonicum* in experimental plots.

Soil nitrate decreases nodule mass and the nitrogen fixation activity of the nodules. Induced mutagenesis of soybean (Carroll et al., 1985) yielded 15 independent nitrate-tolerant symbiotic mutants (*nts* mutants), a phenotype associated with profuse nodulation by *B. japonicum*. In mutant *nts* 382 "supernodulation" occurs with and without nitrate and results from a mutation in a nodulation-regulatory gene, not from inability to use nitrate. Unlike *rjl*, grafting onto reciprocal root stocks of wildtype or other mutants indicated that supernodulation is shoot-controlled.

A dominant *Glycine max* gene, *Rj2*, results in small white nodules when inoculation is made with

two *B. japonicum* serogroups, 122 and C1. Another dominant gene, *Rj*3, conditions small white nodules with *B. japonicum* 33, while the *Rj*4 gene conditions cortical proliferations with *B. japonicum* 61 (see LaRue et al., 1985). These phenotypes can also be generated by random Tn5 mutagenesis of the *Bradyrhizobium* genome (Rostas et al., 1984; Stanley et al., 1986), indicating further that development of effective symbiosis is an interdependent function of both genomes.

The vine *Glycine soja* is the closest ancestral relative of the modern soybean *(G. max).* Both have 40 chromosomes, and they can be intercrossed (Hymowitz & Newell, 1980). The arrangement of leghemoglobin genes in four loci on the *G. soja* chromosome is similar to that in *G. max* (Lee & Verma, 1984). Since it can be effectively nodulated by *B. japonicum* sTI and sTII strains, which nodulate *G. max* cultivars, *Glycine soja* has been used to define symbiotic mutants in *B. japonicum* (Rostas et al., 1984).

Glycine soja and the Chinese *G. max* 'Peking' are also effectively nodulated by fast-growing "soybean" rhizobia isolated in China (Keyser et al., 1982). These rhizobia are generally effective on *Vigna unguiculata* and *Vicia faba* (Broughton et al., 1984; Olson et al., 1984). The mode of inheritance of the nodulation capacity of the host *G. max* cultivars has been elucidated (Devine, 1984) by hybridizing the modern US cultivar 'Kent' with 'Peking' soybean and evaluating F_1 through F_3 progeny for nodulation by a fast-growing strain. 'Kent' and the F_1 hybrids showed rudimentary "pseudonodules," the F_2 fit a 3:1 segregation of pseudonodulated to nodulated plants, and the F_3 fit a 1:2:1 segregation. Hence, 'Peking' carries the recessive allele governing effective nodulation by the fast-growing rhizobia, and 'Kent' carries the dominant allele for exclusion (pseudonodulation). This is not the sole locus governing symbiotic interaction of contemporary soybean cultivars with fast-growing rhizobia.

The fast-growing soybean rhizobia provide useful model systems for the dissection of microbial genes involved in *G. max* symbiosis (Rostas et al., 1984; Olson et al., 1984), but their agricultural importance is minor, for they are neither competitive with the slow-growing *Bradyrhizobium japonicum* for nodulation of commercial soybean cultivars (McLaughlin et al., 1985) nor symbiotically effective on these plants.

Examination of the genome interrelationships among different strains of *B. japonicum* strains establishes a foundation for the molecular genetics of the agronomically important group of strains that nodulate soybean. DNA fragments, used as hybridization probes to genomic Southern blots, have permitted calculation of the degree of DNA sequence divergence in and around major genes from restriction site variation (Stanley et al., 1985). Two groups of strains are defined by highly divergent patterns of conservation of genes for nitrogenase (*nif*DH) and essential nodulation *(nod)* genes (Fig. 1A). One of these is symbiotic genotype I (sTI), e.g., *B. japonicum* USDA 110 (Adams et al., 1984; Kaluza et al., 1983; Lamb & Hennecke, 1986), while a previously undescribed group of strains possesses a second genotype, sTII. Sequence divergence around the nonsymbiotic *recA* and *glnA* genes confirms that sTI and sTII bradyrhizobia are highly divergent for the whole genome. The latter probes define two sTI sublines (Fig. 1A) between which *nif* and *nod* genes may have been transferred. The genotypes sTI and sTII are sufficiently divergent to be described as different bacterial species. These findings correlate with observed phenotypic heterogeneity in the soybean bradyrhizobia. Neither genotype is related to fast-growing PRC rhizobia, which effectively nodulate *Vigna* and Asian soybeans. Thus definition of the species *B. japonicum* on the basis of soybean host-specificity alone is inadequate at the level of molecular genetics. A similar situation may exist with the cowpea group of rhizobia.

IV. Nodule Differentiation: Continuum of Interaction

The host plant can apparently select for early *Sym* genes in a bacterial population. This is evidenced by genetic experiments used to identify the essential nodulation genes of rhizobia. The selection pressure exerted by alfalfa plants selected transconjugant bacteria carrying genetically

complementing *nod* clones in an unreactive Nod⁻ genetic background (Long et al., 1982). It is likely that legume crops can exert similar selection pressure in agricultural environments for lateral transfer of symbiotic genes providing a dynamic for the evolution of *Sym* plasmids in the fast-growing rhizobia.

Development of the root nodule into its fully differentiated form as an intracellular symbiosis is a tightly coordinated process. Certain exopolysaccharide mutants of *R. meliloti* (Finan et al., 1985), although unable to form infection threads, can induce plant cortical cell proliferation characteristic of nodule initiation and form atypical bacteria-free nodules on alfalfa. Similarly, a mutant of *R. japonicum (fredii)* USDA 191 arrests determinate nodule differentiation at the stage subsequent to root-hair curling and infection thread initiation. Nodulelike structures induced by the mutant are amorphous or tumorlike and free of bacteria. Nodulin-35 and leghemoglobin, two nodulins characteristic of fully differentiated nodules, are absent from nodules formed by this Tn5-induced mutant (Stanley et al., 1986). Such bacterial mutations, arresting early root-nodule development, may be useful for the dissection of the plant differentiation pathway, since only a partial spectrum of host nodulins is produced in the nodulelike structures (Stanley et al., 1986).

In a fully developed nitrogen-fixing nodule, the plant exerts decisive influence on bacterial gene expression. The bacteroid hydrogenase activity of *Rhizobium* strain CB756 or 32HI is expressed in nodules formed on *V. unguiculata* and *V. mungo* hosts but suppressed in otherwise effective nodules on *V. radiata* (Gibson et al., 1981). Similar effects of the host plant on *R. leguminosarum* hydrogenase activity has been detected for pea cultivars (Lopez et al., 1983; Bedmar et al., 1983).

Molecular communication between host and microsymbiont occurs early in the rhizosphere and persists throughout endosymbiosis. Random transposition of a transcription fusion vector around the genome of the fast-growing *R. japonicum (fredii)* USDA 201 (Olson et al., 1984) identified three insertion mutants where promoters of genes upstream of an inserted *lac* gene fusion were induced specifically by soybean or *Phaseolus* bean root exudate (host plants of USDA 201) but not those of other legumes. Expression from these promoters is continuous from early events in the rhizosphere to the bacteroid state. "Early" communication is also evidenced in that capsular polysaccharides of some bradyrhizobia are altered within a short time of incubation in the root-exudate of their legume host. Growth on *V. sinensis* root-exudate alters the capsular exopolysaccharide of *Bradyrhizobium (Vigna)* 1001 such that the new sugars are added to native components. Purified *Vigna*-induced rhizobial exopolysaccharide, when added to the uninduced bacteria, enhances the kinetics of the infection process. If nitrate is added to the root system, the host plant root-exudate does not induce changes in the bacterial capsule (Bhagwat & Thomas, 1984).

Genetic dissection of the essential *nod* genes of fast-growing rhizobia indicates that their expression is host-interactive. A small-molecular-weight component of legume root exudates is required with the autoregulatory *nodD* gene to activate transcription of the *nod*ABC genes in *R. meliloti* (Mulligan & Long, 1985) and *R. leguminosarum* (Rossen et al., 1985) and the *nod*FE genes of *R. leguminosarum* (Shearman et al., 1986). This compound has been characterized to be a plant flavone, e.g., luteolin in the case of *R. meliloti* and alfalfa (Peters et al., 1986).

V. Plant Molecular Biology of the Nodule

The requirements for nitrogen fixation are met by the plant through development of an endosymbiotic tissue with characteristic zonation. Bacteria are not found in epidermal or meristematic zones. In determinate nodules, about half the cells of the nodule are infected by rhizobia, and these are interspersed within uninfected cells. These two plant cell types assume different metabolic roles. Thus, the inter- and intracellular compartmentalization seems essential for effective nitrogen fixation and assimilation. Specific nodulin genes are induced in different cell types.

Nodulins have been characterized from soybean (Legocki & Verma, 1981), pea (Bisseling et al., 1983) and alfalfa (Lang-Unasch & Ausubel, 1985). Certain nodulins are apparently synthesized

early in nodule morphogenesis, but the majority (mid-phase and late nodulins) are synthesized concurrently with leghemoglobin (Bisseling et al., 1983; Fuller & Verma, 1984). Messenger RNAs for nodulins, including leghemoglobins, appear and are specifically accumulated in incipient nodule tissue three to five days post-infection, and are fully induced by 10–11 days, prior to the commencement of nitrogenase activity in the bacteroid. Nodulin gene expression is differentially influenced by mutations in rhizobia (Legocki & Verma, 1981), emphasizing the interdependence of the two symbiotic genomes.

STRUCTURAL NODULINS: INTRACELLULAR COMPARTMENTALIZATION *RHIZOBIUM*

Bacteroids within the infected plant cells are each surrounded by peribacteroid membrane (pbm), a compartment derived from the plant plasma membrane during invasion. Its integrity is essential for endosymbiosis and its disintegration or absence renders nodules ineffective (e.g., Werner et al., 1984). The pbm shares many features with the plasma membrane (Verma et al., 1978; Brewin et al., 1985). As the primary symbiotic interface of the root nodule, it is crucial for surface interactions and exchanges of metabolites required in development and maintenance of endosymbiosis.

Several nodule-specific pbm proteins have been characterized. The soybean gene for nodulin-24 (Katinakis & Verma, 1985) encodes a membrane protein that is an integral part of the peribacteroid membrane. Its DNA sequence is interrupted by four intervening sequences (introns). Three coding sequences (exons) and their flanking regions appear to have been generated by duplications of a unit that resembles an insertion element. The units encompass an exon corresponding to each of the three hydrophobic domains in the membrane protein product. Sequence divergence among these repeated units suggests that the gene was generated "recently" during the evolution of symbiotic nitrogen fixation by genome rearrangement(s) possibly resulting from mobilization of the unit during a "pathogenic" state of the endosymbiont. When antiserum is raised against a synthetic peptide corresponding to part of the amino acid sequence from the DNA sequence of the nodulin-24 gene, (Katinakis & Verma, 1985), it identifies a membrane-bound nodulin of the pbm. This protein is not found in the plasma membrane of infected or uninfected cells. Nodule-specific antisera raised against the pbm or the peribacteroid fluid (pbf) internal to it identified several other nodulins by immunoprecipitation of the in vitro translation product of nodule polyA(+) RNA (Fortin et al., 1985). Several clones of pbf and pbm nodulins have recently been isolated (Fortin & Verma, unpublished). Further characterization of these nodulins may reveal the nature of specific modifications that occur to fulfill the functions of specific metabolite transport and segregation of bacteroids from the host cell cytoplasm.

THE METABOLIC NODULINS

Carbon compounds enter the nodule primarily as sugars and the peribacteroid membrane probably restricts sugar transport to C4 dicarboxylic acids, which are the likely carbon sources for bacteroid nitrogen fixation. The leghemoglobin provides facilitated diffusion of oxygen to the bacteroids, and the nitrogenase product, ammonia, diffuses down a gradient created by its assimilation into glutamine by the infected plant cell. A peribacteroid-membrane-associated, ammonia-stimulated ATPase may help in this transport (Blumwald et al., 1985). In legumes forming indeterminate nodules, such as pea, the main compounds transported from the root and nodules are amides (glutamine and asparagine). However, in soybean, cowpea, and other tropical legumes that form determinate nodules, 80% of the organic nitrogen in xylem is accounted for by ureides. Ureide biogenesis from glutamine commences via de novo purine nucleotide biosynthesis, which is enhanced during the onset of nitrogen fixation. A specific enzyme involved in this pathway and essential for nodule-specificity is uricase II encoded by nodulin-35. Similarly, a new sucrose synthetase seems to be

synthesized in nodules and this is a product of the nodulin-100 gene (Thummler & Verma, 1987). Uricase in nodules is specifically localized in the peroxisomes of the uninfected cells (Nguyen et al., 1985).

VI. The Evolution of Nodulins and the Phylogeny of the Leghemoglobin Loci

On the basis of evolutionary conservation, nodulins have been subdivided into proteins common to all legume nodules ("common" or C nodulins) and "species-specific" or S nodulins (Verma & Nadler, 1984). Leghemoglobin, Lb-reductase, certain membrane-bound pbm nodulins, and glutamine synthetase can be assumed to be common nodulins. On the other hand, nodulin-35, xanthine dehydrogenase, and other enzymes for species-specific carbon-nitrogen metabolism in the nodule can be considered as S-nodulins.

Soybean contains four major leghemoglobin proteins, Lba, Lbc1, Lbc2, and Lbc3, which differ slightly in their amino acid sequence. The nucleotide sequences of four soybean Lb genes, two Lb pseudogenes (which do not code for any known Lb protein), and two truncated genes are now known. The genes are typically eucaryotic and their organization within the chromosome has been determined (see Lee & Verma, 1984). The intragenic organization of plant and animal globin genes is very similar, with the difference that the plant genes possess an extra (third) intervening sequence, which occurs in a position separating the coding sequences for two different protein structural units. It has been estimated (Brown et al., 1984) that the plant globins diverged from animal globins over one billion years ago. Such an ancient time of divergence argues against the origin of Lbs by horizontal transfer from animal to legume plant genomes (Lewin, 1981). Instead, globin genes are likely to have been present in the common ancestor of contemporary plants and animals. Indeed, these genes have been found in nitrogen-fixing nonlegume plants (Appleby et al., 1983). The main chromosomal Lb locus contains three Lb genes (*a*, *c*1, and *c*3) and a pseudogene (ψLb); another locus contains three Lb genes (*a*, *c*,1 and *c*3) a pseudogene (ψLb), and another locus contains the Lbc2 gene and a linked pseudogene (ψLb2). Two further chromosomal sites have truncated genes (LbT1 and LbT2). The main locus presumably arose by a series of duplications such as would occur in unequal crossovers. That the intraspecific divergence of Lb genes (within soybean) is less than the interspecific divergence (between say Lba's of soybean and *Phaseolus* bean) indicates a "concerted" evolution of the Lb loci.

A phylogeny of these loci can be established on the basis of their comparative organization in *Phaseolus vulgaris*, *G. soja*, and *G. max* (Lee & Verma, 1984). The kidney bean Lb gene has the same arrangement of introns and exons as *G. max* as well as an extensive DNA sequence homology in its coding and noncoding (5′ and 3′) regions. Since there are four Lb genes in kidney bean, tandem duplication of a single primordial plant globin gene presumably generated four Lb genes in an "Lb locus" prior to the divergence of soybean and kidney bean (Fig. 4). Tetraploidization in *Glycine* would have given the two loci of four genes each and extensive deletion in one of these would have produced the Lbc2 locus (which now contains the Lbc2 and the second pseudo-Lb genes). Other truncated Lb sequences are not linked to the Lb loci in soybean.

VII. Future "Laboratory Evolution" of the Legume Symbiosis

Many breakthroughs have been made in the genetic dissection of the nodulation and nitrogen-fixation genes of the fast-growing rhizobia. Some of these have been extended by recombinant DNA methodologies based on gene homology (e.g., Hahn & Hennecke, 1984) to the bradyrhizobia. In applied science, the primary rationale for the "genetic engineering" of rhizobia has been that the use of *Rhizobium* inoculum is a long-established technology. However, the multifactorial problem

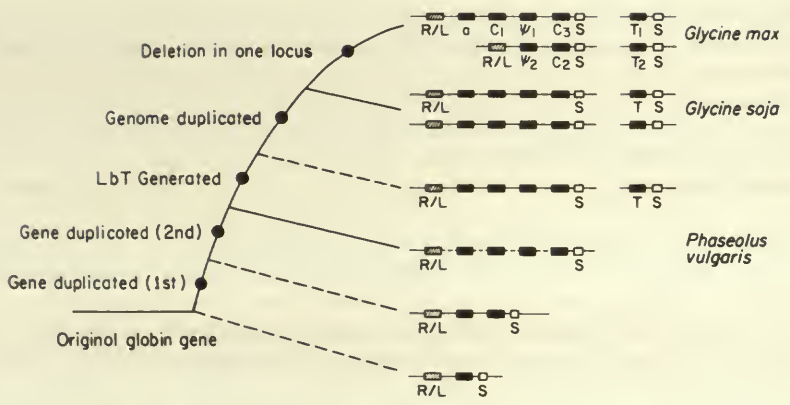

FIGURE 4. Phylogeny of the leghemoglobin loci: chromosomal arrangements during evolution leading to the organization of Lb genes in soybean. T = truncated gene. R/L = sequence expressed in root/leaf. s = repeat element. a = LBa. C_1, C_2, C_3 = LBc_1, LBc_2, LBc_3. ψ_1, ψ_2 = pseudo gene 1 and 2 respectively. (See Lee & Verma, 1984.)

of interstrain competition is a major constraint on the production of a recombinant genotype that can be used as a rhizobial inoculum. Indigenous soil rhizobia, frequently less effective in nitrogen fixation per se, will usually outcompete inoculum strains in nodulation. Several variables (cultivar, interstrain competition factor, soil type) are operative in any field system, and demonstrable success has been achieved in field trials only where the interstrain competition factor is absent. On the other hand, a single alfalfa cultivar derived from phenotypic recurrent selection can give significantly more symbiotic nitrogen fixation than the original material with any of four different *R. meliloti* strains, yielding up to a 17% increase in forage nitrogen (Phillips & Teuber, 1985). This confirms that breeding the host legume could provide a realistic answer to the problem of interstrain competition among rhizobia. The characterization of a nitrate-tolerant "supernodulation" locus in soybean (Carroll et al., 1985) may be an important advance for agricultural legume breeding. Moreover, overall symbiosis-efficiency in all stages of the plant growth may have been selected out of modern legume cultivars since the breeding took place in an environment of high nitrogen soil. This may necessitate a return to the germ plasm.

Many more nodulin genes remain to be characterized: continuing immunocytochemical elucidation of their structure, function, and subcellular localizations is central to our understanding of cellular compartmentalization in endosymbiosis. Characterization of nodulin families and regulation of transcription of nodulin genes would provide significant advances. In general, molecular approaches provide gene probes that may be used to screen legume germ plasm and to monitor the segregation of symbiotic alleles in breeding for legume crop improvement. Whatever the scope or aim of breeding programs, they will ultimately require that the legume and rhizobial combination be bred together, taking account of coevolution in the two genomes. Since several nonlegumes, e.g., *Casuarina, Parasponia,* and *Alnus,* form symbiosis with nitrogen-fixing organisms, it would be interesting to ask how far the genes essential for developing this association have spread in the plant world. By introducing the "essential genes" either through classical genetics or through recombinant DNA approach, it is conceivable that one may be able to develop new associations for harvesting nitrogen for plant growth.

References

Adams, T.H., C. Robertson McClung & B.K. Chelm. 1984. Physical organization of the *Bradyrhizobium japonicum* nitrogenase gene region. J. Bacteriol. 159: 857–862.

Allen, O.N. & E.K. Allen. 1981. The Leguminosae: a Book of Characteristics, Uses and Nodulation. Univ. of Wisconsin Press, Madison, Wisconsin.

Appleby, C.A., J.D. Tjepkema & M.J. Trinick. 1983. Hemoglobin in a non-leguminous plant, *Parasponia*. Possible genetic origin and function in nitrogen fixation. Science 220: 601–606.

Bedmar, E.J., S.A. Edie & D.A. Phillips. 1983. Host plant cultivar effects on hydrogen evolution by *Rhizobium leguminosarum*. Pl. Physiol. 72: 1011–1015.

Bergmann, H., E. Preddie & D.P.S. Verma. 1983. Nodulin-35: a subunit of specific uricase (uricase II) induced and localized in the uninfected cells of soybean nodules. EMBO J. 2: 2333–2339.

Bhagwat, A.A. & J. Thomas. 1984. Legume–*Rhizobium* interactions: host induced alterations in capsular polysaccharides and ineffectivity of cowpea rhizobia. Arch. Microbiol. 140: 260–264.

Bisseling, T., C. Been, J. Klugkist, A. van Kammen & K. Nadler. 1983. Nodule-specific host proteins in effective and ineffective root nodules of *Pisum sativum*. EMBO J. 2: 961–966.

Blumwald, E., M. Fortin, P.A. Rea, D.P.S. Verma & R.J. Poole. 1985. Presence of plasma membrane type H^+-ATPase in the membrane envelope enclosing the bacteroids in soybean root nodules. Pl. Physiol. 78: 665–672.

Brewin, N.J., J.G. Robertson, E.A. Wood, B. Wells, A.P. Larkins, G. Galfre & G.W. Butcher. 1985. Monoclonal antibodies to antigens in the peribacteroid membrane from *Rhizobium*-induced root nodules of pea cross-react with plasma membranes and golgi bodies. EMBO J. 4: 605–611.

Broughton, W.J., N. Heyke, Z.A. Heiner Meyer & C.E. Pankhurst. 1984. Plasmid-linked *nif* and *nod* genes in fast-growing rhizobia that nodulate *Glycine max, Phosphocarpus tetragonolobus* and *Vigna unguiculata*. Proc. Natl. Acad. Sci. U.S.A. 81: 3093–3097.

Brown, G.G., J.S. Lee, N. Brisson & D.P.S. Verma. 1984. The evolution of a plant globin gene family. J. Mol. Evol. 21: 19–32.

Caldwell, B.E. 1966. Inheritance of a strain-specific ineffective nodulation in soybeans. Crop Sci. 6: 427–428.

Carroll, B.J., D.L. McNeil & P.M. Gresshoff. 1985. Isolation and properties of soybean [*Glycine max* (L.) Merr.] mutants that nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci. U.S.A. 82: 4162–4166.

Chakravorty, A.K., W. Zurkowski, J. Shine & B.G. Rolfe. 1982. Symbiotic nitrogen fixation: molecular cloning of *Rhizobium* genes involved in exopolysaccharide synthesis and effective nodulation. J. Mol. Appl. Genet. 1: 585–596.

Crepet, W.L. & D.W. Taylor. 1985. The diversification of the Leguminosae: first fossil evidence of the Mimosoideae and Papilionoideae. Science 228: 1087–1088.

Devine, T.E. 1984. Inheritance of soybean nodulation response with a fast-growing strain of *Rhizobium*. J. Heredity 75: 359–361.

———. 1985. Host range and compatibility of soybean (*Glycine max* L. Merr.) with rhizobial microsymbionts. *In:* R. Shibles (editor), World Soybean Research Conference III: Proceedings. Westview Press, Boulder, Colorado.

Djordjevic, M.A., P.R. Scholfield & B.G. Rolfe. 1985. Tn5 mutagenesis of *Rhizobium trifolii* host-specific nodulation genes results in mutants with altered host range ability. Mol. Gen. Genet. 200: 463–471.

Finan, T.M., A.M. Hirsch, J.A. Leigh, E. Johansen, G.A. Kuldan, S. Deegan, G.C. Walker & E.R. Signer. 1985. Symbiotic mutants of *Rhizobium meliloti* that uncouple plant from bacterial differentiation. Cell 40: 869–877.

Fortin, M.G., M. Zelechowska & D.P.S. Verma. 1985. Specific targetting of membrane nodulins to the bacteroid enclosing compartment in soybean nodules. EMBO J. 4: 3041–3046.

Fuller, F. & D.P.S. Verma. 1984. Appearance and accumulation of nodulin mRNAs and their relationship to the effectiveness of root nodules. Pl. Mol. Biol. 3: 21–28.

Gibson, A.H., B.L. Dreyfus, B.J. Lawn, J.I. Sprent & G.L. Turner. 1981. Host and environmental factors affecting hydrogen evolution and uptake. Pp. 84–96 *in* A.H. Gibson & W.E. Newton (editors), Current Perspectives in Nitrogen Fixation. Elsevier/North Holland, New York.

Hahn, M. & H. Hennecke. 1984. Localized mutagenesis in *Rhizobium japonicum*. Mol. Gen. Genet. 193: 46–52.

Hollis, A.B., W.E. Kloos & G.H. Elkan. 1981. DNA:DNA hybridization studies of *Rhizobium japonicum* and related Rhizobiaceae. J. Gen. Microbiol. 123: 215–222.

Hombrecher, G., R. Gotz, N.J. Dibb, J.A. Downie, A.W.B. Johnston & N.J. Brewin. 1984. Cloning and mutagenesis of nodulation genes from *Rhizobium leguminosarum* TOM, a strain with extended host range. Mol. Gen. Genet. 194: 293–298.

Hubbell, D.H. & G.H. Elkan. 1966. Correlation of physiological characteristics with nodulating ability in *Rhizobium japonicum*. Canad. J. Microbiol. 13:235–241.

Hymowitz, T. & C.A. Newell. 1980. Pp. 251–264 *in* R.J. Summerfield & A.H. Bunting (editors), Advances in Legume Science. Royal Botanic Gardens, Kew.

Jacobs, A.F., M. Zhang, M.G. Fortin & D.P.S. Verma. 1986. Several nodulines of soybean share structural domains but differ in their subcellular locations. Nucl. Acids Res. 15: 1271–1280.

Jordan, D.C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen nov., a genus of slow-growing, root-nodule bacteria from leguminous plants. Intl. J. System. Bacteriol. 32: 136–139.

Kaluza, K., M. Fuhrmann, B. Hahn, B. Regensburger & H. Hennecke. 1983. In *Rhizobium japonicum* the nitrogenase genes *nifH* and *nifDH* are separated. J. Bacteriol. 155: 915–918.

Katinakis, P. & D.P.S. Verma. 1985. Nodulin-24 gene of soybean codes for a peptide of the peribacteroid membrane and was generated by tandem duplication of sequence resembling an insertion element. Proc. Natl. Acad. Sci. U.S.A. 82: 4157–4161.

Keyser, H.H., B.B. Bohlool, T.S. Hu & D.F. Weber. 1982. Fast-growing rhizobia isolated from root nodules of soybean. Science 215: 1631–1632.

Lamb, J.W. & H. Hennecke. 1986. In *Bradyrhizobium japonicum* the common nodulation genes *nodABC* are linked to *nifA* and *fixA*. Mol. Gen. Genet. 202: 512–517.

Lang-Unnash, N. & F.M. Ausubel. 1985. Nodule-specific polypeptides from effective alfalfa root nodules and from ineffective nodules lacking nitrogenase. Pl. Physiol. 77: 833–839.

Lara, M., J.V. Cullimore, P.J. Lea, B.J. Miflin, A.W.B. Johnston & J.W. Lamb. 1983. Appearance of a novel form of plant glutamine synthetase during nodule development in *Phaseolus vulgaris* L. Planta 157: 254–258.

LaRue, T.A., B.E. Kneen & E. Gartside. 1985. Plant mutants defective in symbiotic nitrogen fixation. Pp. 39–48 *in* R. Marcellin (editor), Analysis of Plant Genes Involved in the Legume–*Rhizobium* Symbiosis. OECD, Paris.

Lee, J.S. & D.P.S. Verma. 1984. Structure and chromosomal arrangement of leghemoglobin genes in kidney bean suggest divergence in soybean leghemoglobin gene loci following tetraploidization. EMBO J. 3: 2745–2752.

Legocki, R.P. & D.P.S. Verma. 1980. Identification of "nodule-specific" host proteins (nodulins) involved in the development of *Rhizobium*–legume symbiosis. Cell 20: 153–163.

_____ & _____. 1981. Multiple immunoreplica technique: Screening for specific proteins with a series of different antibodies using one polyacrylamide gel. Anal. Biochem. 111: 385–392.

Lewin, R. 1981. Evolutionary history written in globin genes. Science 214: 426–429.

Lie, T.A. 1978. Symbiotic specialization in pea plants: the requirement of specific *Rhizobium* strains for peas from Afghanistan. Ann. Appl. Biol. 88: 462–465.

_____.1984. Host genes in *Pisum sativum* L. conferring resistance to European *Rhizobium leguminosarum* strains. Pl. & Soil 82: 415–425.

_____ & D. Goktan. 1984. Gene centres, a source of genetic variants in symbiotic nitrogen fixation: the symbiotic response of the cultivated pea to *Rhizobium leguminosarum* strains from Europe and the Middle East. Pl. & Soil 82: 359–367.

Long, S.R., W.J. Buikem & F.M. Ausubel. 1982. Cloning of *Rhizobium meliloti* nodulation genes by direct complementation of Nod^- mutants. Nature (London) 298: 485–488.

Lopez, M., V. Carbonero, E. Cubrera & T. Ruiz-Argueso. 1983. Effects of host on the expression of the H_2 uptake of *Rhizobium* in legume nodules. Pl. Sci. Lett. 29: 191–199.

McComb, J.A., J. Elliot & M.J. Dilworth. 1975. Acetylene reduction by *Rhizobium* in pure culture. Nature 256: 409–410.

McLaughlin, T.J., P.A. Owens & S.G. Alt. 1985. Competition studies with fast-growing *Rhizobium japonicum* strains. Canad. J. Microbiol. 31: 220–223.

Mulligan, J.T. & S.R. Long. 1985. Induction of *Rhizobium meliloti nodC* expression by plant root exudate requires *nodD*. Proc. Natl. Acad. Sci. U.S.A. 82: 6609–6613.

Nguyen, T., M. Zelechowska, V. Foster, H. Bergmann & D.P.S. Verma. 1985. Primary structure of the soybean nodulin-35 gene encoding uricase II localized in the peroxisomes of uninfected cells of nodules. Proc. Natl. Acad. Sci. U.S.A. 82: 5040–5044.

Olson, E., M. Sadowksy & D.P.S. Verma. 1984. Identification of genes involved in the *Rhizobium*–legume symbiosis by Mu-dI (Kan, lac)-generated transcription fusions. Bio/Technology 3: 143–149.

Pagan, J.D., J.J. Child, W.R. Snowcroft & A.H. Gibson. 1975. Nitrogen fixation by *Rhizobium* cultured on a defined medium. Nature 256: 406–407.

Peters, N.K., J.W. Frost & S.R. Long. 1986. A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233: 977–980.

Philips, D.A. & L.R. Teuber. 1985. Genetic improvement of symbiotic nitrogen fixation in legumes. Pp. 11–17 *in* H.J. Evans, P.J. Bottomley & W.E. Newton (editors), Nitrogen Fixation Research Progress. Martinus Nijhoff Publishers, Dordrecht.

Rolfe, B.G. & J. Shine. 1984. *Rhizobium*–Leguminosae symbiosis: the bacterial point of view. Pp. 95–128 *in* D.P.S. Verma & T. Hohn (editors), Genes Involved in Microbe–Plant Interactions. Springer-Verlag, Vienna & New York.

Rossen, L., C.A. Shearman, A.W.B. Johnston & J.A. Downe. 1985. The *nodD* gene of *Rhizobium leguminosarum* is autoregulatory and in the presence of plant exudate induces the *nodABC* genes. EMBO J. 4: 3369–3373.

Rostas, K., P.R. Sista, J. Stanley & D.P.S. Verma. 1984. Transposon mutagenesis of *Rhizobium japonicum*. Mol. Gen. Genet. 197: 230–235.

Ruvkun, G.B. & F.M. Ausubel. 1980. Interspecies homologies of nitrogenase genes. Proc. Natl. Acad. Sci. U.S.A. 77: 113–118.

Schmidt, J., M. John, E. Kondorosi, A. Kondorosi, U. Weineke, G. Schroder, J. Schroder & J. Schell. 1984. Mapping of the protein coding regions of *Rhizobium meliloti* common nodulation genes. EMBO J. 3: 1705–1711.

Shearman, C.A., L. Rossen, A.W.B. Johnston & J.A. Downie. 1986. The *Rhizobium leguminosarum* nodulation gene *nodF* encodes a polypeptide similar to acyl-carrier protein and is regulated by *nodD* plus a factor in pea root exudate. EMBO J. 5: 647–652.

Sprent, J.I. 1981. Functional evolution in some papilionoid root nodules. Pp. 671–676 *in* R.M. Pollhill & P.H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Stanley, J., G.G. Brown & D.P.S. Verma. 1985. Slow-growing *Rhizobium japonicum* comprises two highly divergent symbiotic types. J. Bacteriol. 163: 148–154.

———, D. Longtin, C. Madzrak & D.P.S. Verma. 1986. Genetic loci in *Rhizobium japonicum (fredii)* affecting soybean root-nodule differentiation. J. Bact. 166: 628–634.

Thummler, F. & D.P.S. Verma. 1987. Nodulin-100 of soybean is the subunit of sucrose synthetase regulated by the availability of free heme in nodules. J. Biol. Chem. (in press).

Verma, D.P.S. & K. Nadler. 1984. Legume–*Rhizobium* symbiosis: host's point of view. Pp. 58–94 *in* D.P.S. Verma & T. Hohn (editors), Genes Involved in Microbe–Plant Interactions. Springer-Verlag, Vienna & New York.

———, V. Kazazian, V. Zogbi & A.K. Bal. 1978. Isolation and characterization of the membrane envelope enclosing the bacteroids in soybean root nodules. J. Cell Biol. 78: 919–936.

———, M.G. Fortin, J. Stanley, V. Mauro, S. Punohit & N. Morrison. 1986. Nodulins and nodulin genes of *Glycine max*. Pl. Mol. Biol. 7: 51–61.

Werner, D., E. Morschel, R. Kort, R.B. Mellor & S. Bassarab. 1984. Lysis of bacteroids in the vicinity of the host cell nucleus in an ineffective (fix$^-$). Planta 162: 8–16.

Williams, L.F. & D.L. Lynch. 1954. Inheritance of a non-nodulating character in soybean. Agron. 46: 28–29.

Winarno, R. & T.A. Lie. 1979. Competition between *Rhizobium* strains in nodule formation: interaction between nodulating and non-nodulating strains. Pl. & Soil 51: 135–142.

Young, J.P.W., A.W.B. Johnston & N.J. Brewin. 1982. A search for peas (*Pisum sativum* L.) showing strain specificity for symbiotic *Rhizobium leguminosarum*. Heredity 48:197–201.

STRUCTURE AND FUNCTION OF ROOT NODULES FROM WOODY LEGUMES

J. I. Sprent,[1] J. M. Sutherland[1] and S. M. de Faria[1]

Abstract

Woody papilionoid legumes show varied nodule morphology. Two forms have particular interest for nodule evolution. First, the aeschynomenoid type found commonly in the tribes Dalbergieae and Aeschynomeneae. Such nodules are associated with lateral roots and follow infections at the lateral-subtending root junction. Infection threads are rarely, if ever, found and the infected tissue does not contain uninfected (interstitial) cells. Second, some primitive genera of the tribes Dalbergieae, Robinieae, and Millettieae have woody, indeterminate nodules. In these, infection threads are formed only when the bacteria attempt to penetrate host cells. These threads proliferate within the host cell and persist throughout the active nitrogen-fixing period. Similar infected cells have been found in all eight genera so far examined in the Caesalpinioideae and are considered to be a primitive case. They have not been found in mimosoid nodules. Primitive nodules often contain numerous massive vascular bundles with many thick-walled xylem elements. Many extant legumes lack root hairs; this is also thought to be a primitive feature. A hypothesis is developed for the evolution of legume nodules from an ancestor which lacked root hairs and which did not contain bacteria within infection threads in the early stages of infection. Infection of legumes via root hairs is an advanced character. An attempt is made to accommodate the data and hypothesis into a taxonomic framework.

I. Introduction

In the last five years, there has been a major effort to check for nodulation among woody legumes and to identify those species of potential significance for practical use in tropical countries, especially where the climate is arid or semiarid. Many new records of nodulation have been made and rhizobial strains isolated. Studies of the structure of nodules on woody legumes are few (e.g., Lawrie, 1983a; Spratt, 1919). The pioneering work of Corby (e.g., 1981), linking nodule morphology with legume taxonomy, has laid the foundation for considering structure and function in taxonomic terms. A study restricted to some advanced papilionoid tribes (Sprent, 1980, 1981) suggested that this approach can be useful. This report presents new data drawn from a number of woody legume tribes.

II. Materials and Methods

Sources of material

African samples were taken from material collected by Corby, fixed in formalin:acetic:alcohol, and preserved in alcohol; nitrogen-fixing activity was generally judged by pink internal nodule coloration. Brazilian material was fixed in glutaraldehyde; nitrogen-fixing activity was checked using the acetylene reduction assay (see Faria et al., 1984). Other specimens were taken from plants grown in the greenhouse in Dundee: seeds and rhizobia were from various countries. For location of voucher material, see Faria et al. (1984, 1987a) and Corby (1974).

[1]Department of Biological Sciences, University of Dundee, Dundee, DD1 4HN, Scotland, U.K.

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MICROSCOPIC TECHNIQUES

For examination of vascular tissues and formation of sclereid layers, nodules were cleared in sodium hydroxide solution, followed by hydrogen peroxide. Glutaraldehyde fixed material was examined by scanning electron and transmission electron microscopy and light microscopy. Details of these methods can be found in Sutherland & Sprent (1984), with the exception that sectioned material was first embedded in LR white, rather than Spurrs resin.

III. Observations

Nodules on woody legumes fall into two categories: short-lived and presumably renewed each growing season or more often; and longer-lived, usually perennial.

SHORT-LIVED AESCHYNOMENOID NODULES

These nodules appear to be confined to two tribes, the Aeschynomeneae and the Dalbergieae.

Aeschynomeneae

The genera of this tribe are not all woody, and the two that have been studied in most detail. *Arachis* and *Stylosanthes*, are herbaceous. They are characterized by the unusual feature that the endosymbiont *(Rhizobium)* enters its host, not by the familiar root hair infection process (Fig. 1), but via breaks in the root surface associated with the emergence of lateral roots. A similar mode of entry, via wounds, is adopted by the closely related agrobacteria, which cause crown and hairy root galls. The macroscopic appearance of such nodules is illustrated in Figure 5. A transverse section of a functional nodule of this type is shown in Figure 6. The salient features of *Arachis* and *Stylosanthes* nodules are listed in Table 1.

We have examined several other genera in this tribe (Table 2), some herbaceous and some woody, and they all conform to the general pattern of *Arachis*/*Stylosanthes* with minor variations. Where examined in sufficient detail, nodule vascular traces have always been found to be connected to the subtending root. Often this root was very woody, sometimes clearly showing several seasons of growth. Nodules were occasionally grouped at the point of emergence of the lateral root, and their cortices were fused with either/or both of those of the lateral and subtending roots. Infected cells were always nonvacuolate, but bacteroids varied in shape from nearly spherical to definitely rod-shaped. Root hairs were either absent or infrequent.

On the basis of nodule structure and association with lateral roots, we agree with Corby (1981) that there is a great deal of uniformity within the tribe Aeschynomeneae. We shall therefore take as a working hypothesis that infection, as established for *Arachis* and *Stylosanthes*, occurs via lateral root junctions. This route bypasses the specific recognition processes known to be involved in root hair infection (Bauer, 1981). Is this a sign of primitiveness or advancement? Corby (1981) considered aeschynomenoid nodules to be advanced by reason of their reduced size and other attributes. In the classification of papilionoid legumes of Polhill & Raven (1981) the Aeschynomeneae are also thought to be relatively advanced, for reasons including their tendency toward an herbaceous habit.

The woody genera *Aeschynomene* and *Sesbania* both produce nodules on stems, especially when grown under waterlogged conditions (which tend to suppress nodulation of roots). The structure of stem nodules in *Aeschynomene* is similar to that of root nodules on the same plant, even to the extent of being associated with adventitious root initials (Vaughn & Elmore, 1985; Yatazawa et al., 1984; Yatazawa & Susilo, 1980). The situation in *Sesbania* is more complex. Although root nodules are

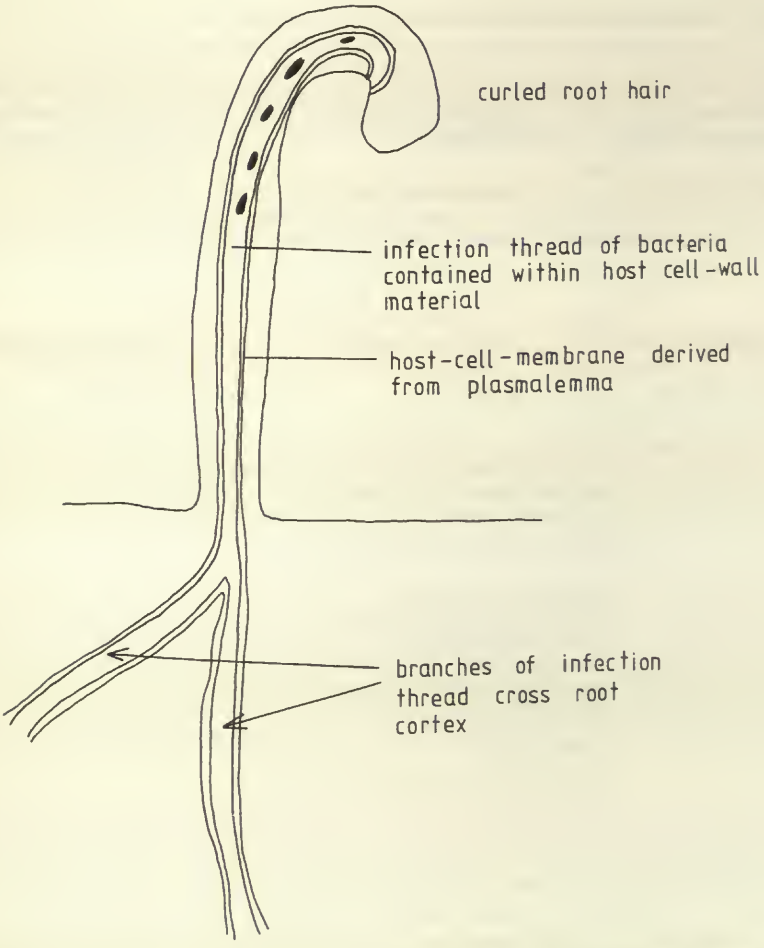


FIGURE 1. Diagram illustrating an infection thread containing rhizobia passing down a root hair to the cortex. This situation occurs (with slight variations) in advanced papilionoid legume tribes such as the Trifolieae, Vicieae, and Phaseoleae.

found close to the junctions of lateral roots, they are formed following root hair infection (Harris et al., 1949; Olsson & Rolfe, 1985). In common with all other nodules infected in this way, their infected tissue contains a mixture of infected and uninfected cells. Morphologically, young nodules have an aeschynomenoid appearance, but they may later become lobed and even give rise to rootlets (Harris et al., 1949). Older root nodules have well-developed sclerenchyma external to the vascular bundles. Stem nodules, on the other hand, may be formed by a different strain of *Rhizobium* than root nodules; infection is via breaks and is associated with adventitious root formation (Tsein et al., 1983). Infection threads may be formed later in nodule development (Duhoux, 1984; Olsson & Rolfe, 1985). Thus, although superficially the nodules on stems and roots of *Aeschynomene* and *Sesbania* have much in common, they differ in the mode of infection of root nodules and the occurrence of infection threads. *Sesbania* on some criteria appears to be closely related to the Aeschynomeneae, but the nodule data in general are consistent with its inclusion in the Robinieae (see Polhill & Sousa, 1981). *Erythrina* (Phaseoleae) nodules were reported, on the grounds of shape

TABLE 1. Comparison of infection and nodulation in *Arachis* and *Stylosanthes*.

Character	*Arachis*	*Stylosanthes*
Point of infection	at junctions of lateral roots	at junctions of lateral roots
Penetration of root cortex	intercellular	by invading cells which subsequently collapse
Formation of nodules	by repeated divisions of invaded cells (not collapsed) of inner cortex, giving an infected region approximately uniform in cell age and with no interstitial (uninvaded) cells	same as *Arachis*
Structure of mature, N-fixing cells	nonvacuolate, bacteroids spherical	vacuolate, bacteroids more variable in shape
Vascular connections	to branch root	to main root

TABLE 2. Species of the tribe Aeschynomeneae which have been examined for nodule structure in the present study (except where specified). Material from the collection of Corby is now housed in Dundee.

Aeschynomene rhodesiaca
Arachis hypogea (Chandler, 1978)
Humularia megalophylla
Kotschya strobilata
Ormocarpum kirkii
Ormocarpum sennoides
Smithia confecta
Stylosanthes spp. (Chandler et al., 1982)
Zornia milneana

and association with lateral roots to be aeschynomenoid (Faria et al., 1984). However, internal structure shows interstitial cells and infection threads, features which do not conform to our revised definition of the aechynomenoid nodule. Further, they contain calcium oxalate crystals, which are typical of determinate, ureide-exporting species of the Phaseoleae (Sutherland & Sprent, 1984).

Nodules associated with lateral roots and probably infected via epidermal breaks may occur elsewhere, for example in *Viminaria denudata* (Mirbelieae) (Fraser, 1931) and *Neptunia oleracea*, *Mimosa scabrella*, and probably other members of the Mimosoideae and some Caesalpiniodeae (unpublished observations from our laboratory), as well as in the Dalbergieae. Except in the latter, however, infection threads and interstitial cells are found, so that they do not conform to the aeschynomenoid category (see section IV below).

Dalbergieae

In a recent survey of newly recorded nodules on Brazilian trees, Faria et al. (1984, 1987a) noted that most were associated with lateral roots. We have now examined more genera in greater detail. Table 3 lists these genera, and where nodules are associated with lateral roots, have limited growth and uniform infected tissue, they are classified as aeschynomenoid. Preliminary studies of sections of such nodules show all the characteristics described in Table 3, with minor variations in exact position with respect to branch roots and origin of vascular traces. The genus *Pterocarpus* appears to nodulate in Africa but not in Brazil. A similar situation exists in *Parkia* (Mimosoideae). Both genera are cytologically diverse (Goldblatt, 1981) and may repay more detailed study. Nodules on

TABLE 3. Nodule morphology of members of the Dalbergieae. Material from Faria et al. (1984, 1987a) and Faria et al. (1985) or from examination of specimens from Corby's collection. Aeschynomenoid here denotes globose to oblate, associated with a lateral root and a uniformly infected central tissue. Bacteroid morphology varies from globose to rod-shaped.

Species	Nodule type
Andira[1] (7 species)	astragaloid
Centrolobium (4 species)	aeschynomenoid
Dalbergia (12 species)	aeschynomenoid
Geoffroea decorticans	aeschynomenoid[2]
Hymenolobium alagoanum var. *parvifolium*	astragaloid
Machaerium (14 species)	aeschynomenoid
Platymiscium floribundum	aeschynomenoid
Platypodium elegans	aeschynomenoid
Pterocarpus angolensis[3]	aeschynomenoid
brenanii	
macrocarpus	
Tipuana tipu	aeschynomenoid

[1]Roots of *Andira* were often very hairy and nodules up to 5 cm (Faria et al., 1986).

[2]Association with lateral roots not always clear; nodules often paired about a lateral root in such a way that the root appeared to grow out of the nodule. These are being examined in more detail.

[3]One specimen recorded as branched by Corby. Some examined here were paired, as in *Goeffroea*. No nodules have been found on Brazilian species of this genus.

Andira and *Hymenolobium* are indeterminate (i.e., have a long-lived apical meristem and interstitial cells in their infected regions), and they have a number of other distinguishing features. One of these is the continuous layer of sclereids (stone cells) in the nodule cortex (Fig. 7). Some of these stone cells appear to have tanninlike contents. No such sclereids have been found in nodules of other genera of the Dalbergieae.

In her account of wood anatomy as a possible taxonomic tool, Baretta-Kuipers (1981) separated four genera of the Dalbergieae as atypical *(Andira, Hymenolobium, Vatairea, Vatairiopsis)* on the grounds of a much coarser structure, larger vessels, and different arrangement of rays. She suggested that these genera may be closer to the Sophoreae than the Dalbergieae. We have examined two of these four genera (Faria et al., (1984, 1987a) could find no nodules on *Vatairea* or *Vatairiopsis*), and agree that on the grounds of nodule morphology and the development of the sclereid layer, there are similarities with some of the Sophoreae. *Andira* and *Hymenolobium* show copious production of root hairs; genera in the Dalbergieae, which have aeschynomenoid nodules, show varied numbers of root hairs. The most outstanding structural feature of nodules on these two genera is that they confine their rhizobia within host-cell wall material (persistent infection threads) during the active nitrogen-fixing stage (Faria et al., 1986; Faria et al., 1987b). The possible significance of this feature will be considered in section IV.

PERENNIAL NODULES ON OTHER WOODY PAPILIONOID LEGUMES

The study has so far concentrated on members of the Millettieae and a few genera of the Sophoreae. One of the characters looked for was the presence of sclereids (Table 4). Taxonomically,

TABLE 4. Nodules on woody legumes found to have extensive sclereid production—at least one continuous layer of such cells in the cortex, sometimes several. Sclereids were more or less isodiametric and tightly packed, making nodules very brittle. A large number of other genera from each of the tribes listed was examined and found not to contain sclereids. All data from this investigation except where specified.

Species	Tribe
Andira inermis and other species	Dalbergieae
Baphia nitida	Sophoreae
Bowdichia virgiloides	Sophoreae
Dahlstedtia pinnata	Millettieae
Derris guilleminianus	Millettieae
Hymenolobium alagoanum	Dalbergieae
Inga capitata	Ingeae
Laburnum sp.	Genisteae
Leucaeana leucocephala	Acacieae; Allen & Allen, 1981
Lonchocarpus nelsii	Millettieae
Millettia stuhlmannii	Millettieae
Ormosia arborea	Sophoreae
Ostryocarpus stuhlmannii	Millettieae
Sesbania grandiflora	Robinieae; Harris et al., 1949
Wisteria sinensis	Millettieae; Jimbo, 1927

Millettia, Lonchocarpus, and *Derris* (all Millettieae) are considered to be very similar (Geesink, 1984), and this is borne out by the presence of sclereids, consistent with the past inclusion of these genera in the Dalbergieae (Geesink, 1981). The presence of sclereids in *Ostryocarpus (Xeroderris)* formerly (Millettieae) also suggests an affinity with this group. On the basis of wood anatomy (Baretta-Kuipers, 1981) and general nodule structure, these genera form a group with *Andira* and *Hymenolobium* from the Dalbergieae. However, unlike the last two genera, *Lonchocarpus* and *Derris* (*Millettia* has not yet been examined) do not have persistent infection threads. Within the Millettieae, only *Cyclolobium* and *Dahlstedtia* (both of which Geesink (1981) placed at the base of the tribe, near the Dalbergieae and Robinieae) have been found to contain these structures.

The genus *Poecilanthe* was placed in this manuscript in the Robinieae (following Geesink, 1984), although the nodule structure of *Poecilanthe* does not conform to that of other genera in the tribe (Faria et al., 1987b). Lavin (1987) suggested that *Poecilanthe* should be placed in the Dalbergieae rather than the Robinieae. Our observations are in agreement with this suggestion, since *Andira* and *Hymenolobium* (Dalbergieae) have nodules similar in structure to those of *Poecilanthe.*

The Sophoreae are a rather disparate group (Polhill, 1981a), intermediate between the Papilionoideae and the Caesalpinioideae. Geesink's (1981) suggestion that *Ormosia* is allied to *Millettia* is borne out by nodule structure. The sclereids in *Bowdichia,* although frequent and prominent, were thinner-walled than those of *Ormosia.* We are currently examining the structure of infected cells of this group in detail, and preliminary evidence agrees with the suggestion of Stirton (pers. comm.) and others that this tribe is polyphyletic. Extensive sclereid production has occasionally been reported in the literature in older root nodules of *Sesbania* (Harris et al., 1949) and *Wisteria sinensis* (Millettieae) (Jimbo, 1927), as well as in mimosoid nodules (*Inga,* this study; *Leucaena leucocephala,* Allen & Allen, 1981). Among the woody papilionoid and mimosoid species, therefore, sclereid production is quite common. However, it appears to be sporadic in herbaceous species and possibly related to growth in dry areas (e.g., *Kennedya prostrata* and *Hardenbergia comptoniana,* both members of the Phaseoleae). In the caesalpinioid nodules, only occasional sclereids have so far been found.

Many of these woody nodules have cell inclusions with staining properties and appearance suggestive of polyphenols and flavonoids in cells of their cortices. In view of the taxonomic

significance of oxidation states of flavonoids (Gottlieb et al., quoted by Geesink, 1981) and the relationship between flavonoid and lignin biochemistry, the association between nodule flavonoids and sclereids, and plant wood anatomy is worthy of further investigation, especially since production of flavonoids has been shown to be associated with nodule effectiveness in *Lotus* (Pankhurst & Jones, 1978).

VASCULAR BUNDLES IN PERENNIAL NODULES

In many herbaceous legumes the vascular network, although extensive, has rather slender individual bundles. For example, in *Vigna* the entire cross-sectional area of a bundle is similar to that of a single cortical cell (Sutherland & Sprent, unpublished). In the present study, one of the most striking structural features was the variation in diameter and branching pattern of the vascular systems. The functional significance of these differences is not yet clear, but the variations in xylem content, from a few elements per bundle (Fig. 8) to 10–20 or more (Fig. 9), are likely to affect transport. One possibility is that where nodules have extensive surface cork and/or sclereids in the cortex, direct exchange of nutrients with soil solution might be restricted so that all supplies would have to reach the nodule through the vascular system. For essential elements such as calcium, xylem transport is more likely than phloem or an intercellular route (see discussion in Raven, 1986). Harris et al. (1949) also considered sclereid production and numerous vascular traces to have functional significance, in this case associated with perennial growth. Cork production may limit gaseous exchange and hence maximum potential nitrogenase activity, but in dry areas reduced water loss may offset this disadvantage (Sprent, 1985).

STRUCTURE OF INFECTED TISSUE

Apart from aeschynomenoid nodules described above, the woody nodules examined here and by others such as Spratt (1919), Harris et al. (1949), and Lawrie (1983a), all have a mixture of infected and uninfected (interstitial) cells as illustrated by Lawrie (1983a). The proportion of the two types varies widely, but whether this is a host or rhizobial conditioned feature is not yet known. It is likely to result in large differences in nitrogen-fixing efficiency (see also Lawrie, 1983b). The infected cells of most species examined were nonvacuolate and the bacteroids more or less rod shaped, similar in size to those of soybean (see Sprent, 1980, for associated factors). With the exceptions noted above *(Andira, Hymenolobium, Cyclolobium, Dahlstedtia,* and *Poecilanthe),* all those examined in sufficient detail form infection threads at some stage prior to the commencement of nitrogen fixation and release bacteria from these threads in the final stages prior to the onset of nitrogenase activity (Figs. 2, 11, 13).

CAESALPINIOID NODULES

Occurrence of nodules

Recent studies have added several more genera to those known to be nodulated (Faria et al., 1984, 1986, and references therein). The current situation is summarized in Table 5. The most numerous confirmed reports of nodulation are those in the tribe Caesalpinieae, Table 6A. (Table 6B lists species lacking nodules in this tribe.) We have studied the nodule structure in species from eight nodulated genera and found them to be strikingly similar (see below). In the Cassieae, all the confirmed reports of nodulation are from species now separated into the genus *Chamaecrista* (Irwin & Barneby, 1982), although the name *Cassia* for some of them is still often used. Within the

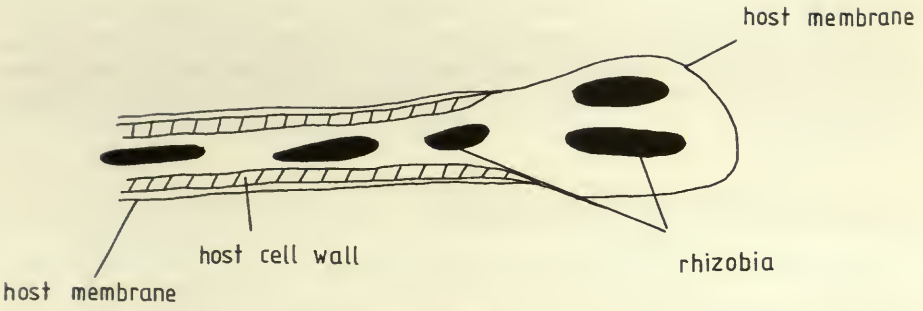


FIGURE 2. Diagram illustrating how rhizobia are released from the confines of the infection thread during the formation of active nitrogen-fixing cells in legumes of the tribes Trifolieae, Vicieae, and Phaseoleae. (See also Figs. 11, 13.)

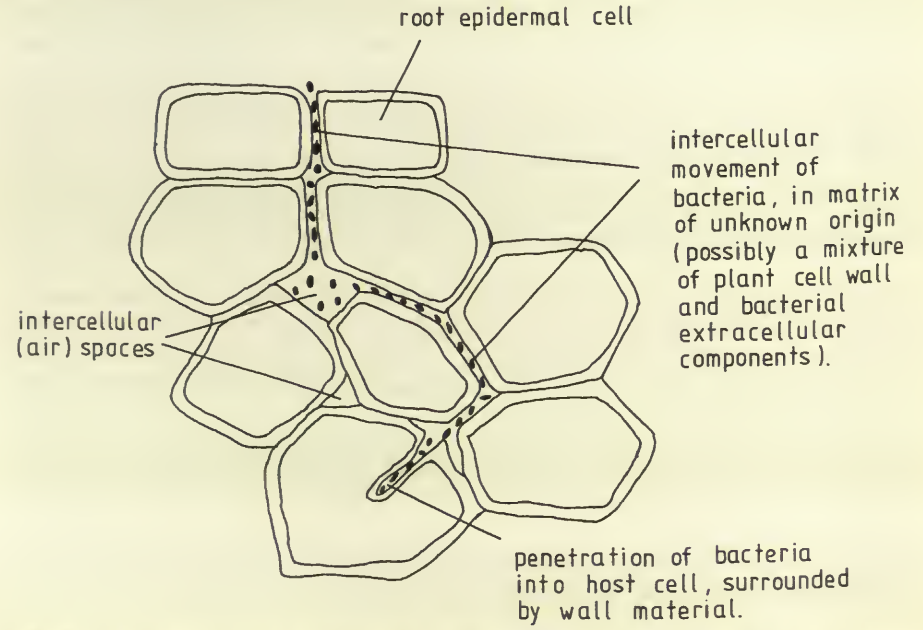
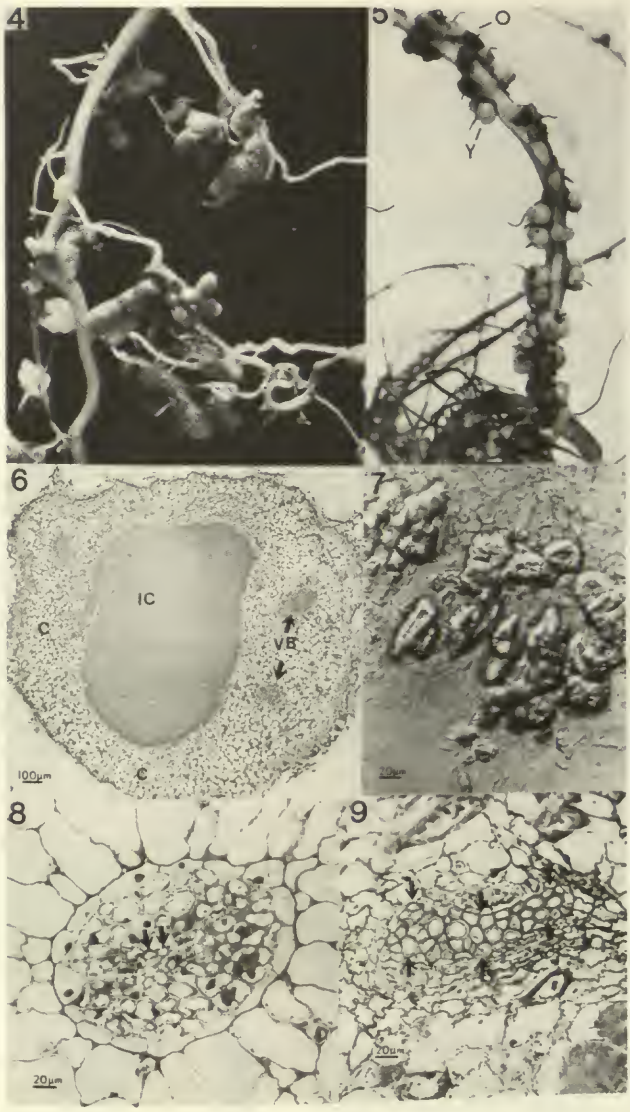
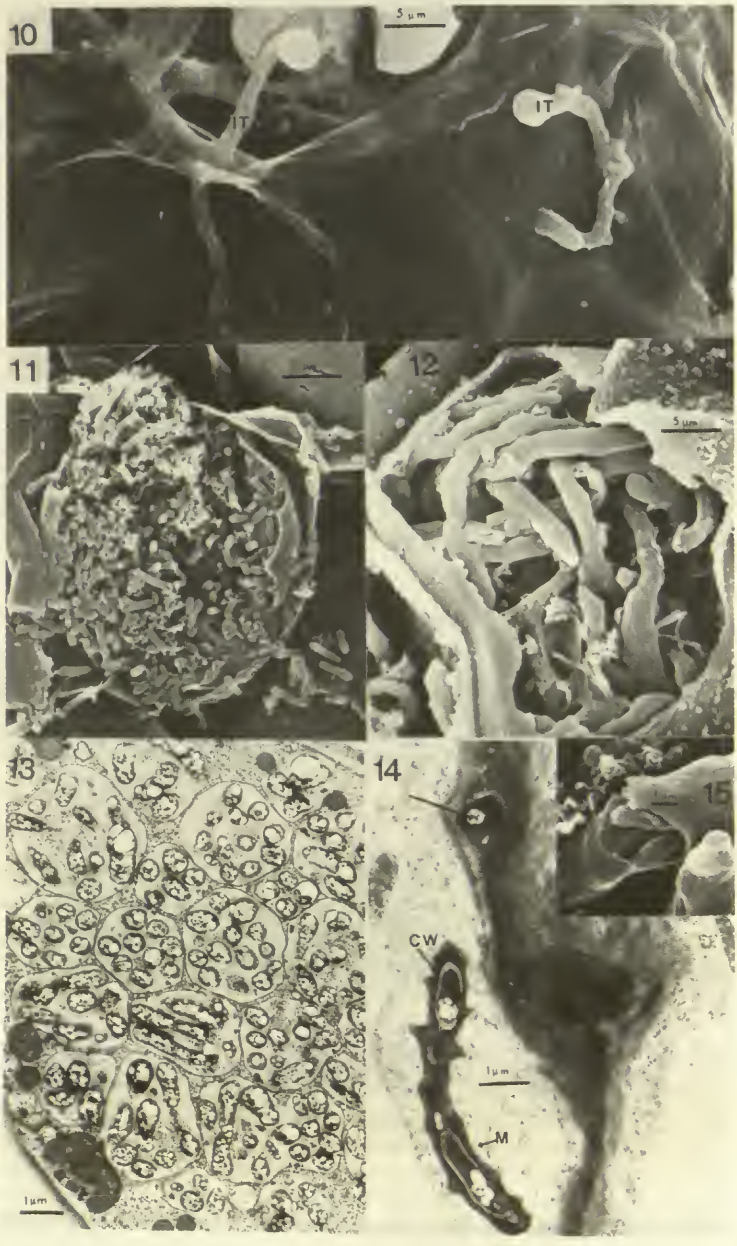


FIGURE 3. Diagram illustrating the intercellular passage of rhizobia, which may occur in many primitive legumes. Infection threads are only formed at the point of entry into the host cell. (See also Figs. 12, 14, 15.)

Cercideae, there are no confirmed reports of nodulation. [Note: *Griffonia* quoted as nodulated in Sprent & Raven (1985) was an error of the senior author.] The situation in the Detarieae (Table 7A, B) is far from clear. Of the eight genera reported as having nodulated species, five are doubtful and three are based on only one or two reports, albeit from very sound sources (Allen & Allen, 1981; Norris, 1959). More observations are urgently needed on this group. Occasional reports have been made on nodulation and even more rarely on nitrogenase activity in species from the Amherstieae. For example, Whitton (1962) found two species of *Dicymbe* to be abundantly nodulated,



FIGURES 4–9.—4. Nodulated root system of *Prosopis juliflora*. All mimosoid, caesalpinioid, and those papilionoid genera with persistent infection threads have nodules of this general type, showing indeterminate growth, sometimes with branching and distinct apical meristems (AM).—5. Nodulated root system of *Dalbergia* sp. Note the association of nodules with lateral roots and the presence of both old (O) and young (Y) nodules on the same root.—6. Transverse section of a nodule of *Dalbergia nigra* showing uniform central infected tissue (IC), typical of aeschynomenoid nodules. C = cortex, VB = vascular bundle.—7. Thick-walled sclereids from the cortex of a nodule of *Ormosia arborea*.—8. Vascular bundles in a nodule of *Isotropis cuneifolia*. Advanced papilionoid nodules have bundles of a similar type, with only a few xylem elements (arrows).—9. Vascular bundles in a nodule of *Ormosia arborea*. Many nodules of the primitive woody legumes have massive bundles of this type, with many thick-walled xylem elements (arrows). The exact arrangement of xylem and phloem varies somewhat.



FIGURES 10–15.—10. Infection threads (IT) passing from cell to cell in the cortex of a nodule of *Galega orientalis*: this is the typical situation following root hair infection (Fig. 1). One or more files of rhizobia are found in such infection threads.—11. An infected cell of a nodule of *Pterocarpus macrocarpus* showing numerous nitrogen-fixing bacteroids not confined by infection threads. The host cell membrane, seen by transmission electron microscopy (Fig. 13), is not normally visible under the scanning electron microscope.—12. Persistent infection threads in a nodule of *Tachigali paniculata* var. *alba*. These form following a process as illustrated in Figure 3. Note that the diameter of these threads is similar to that shown in Figure 10.—13. Infected cell of an advanced legume *(Glycine max)*. Nitrogen-fixing bacteroids are surrounded only by membrane of host cell origin.—14. Persistent infection thread in a nodule of *Poecilanthe parviflora*, bounded by both host cell wall material (CW) and host membrane (M), as shown in Figure 2. Also shown is intercellular passage of a bacterial cell (compare Fig. 3).—15. A broken, persistent infection thread in a nodule of *Andira* sp. showing several files of rhizobia.

TABLE 5. Summary of the reports of nodulation in the Caesalpinioideae. Values in brackets followed by ? indicate the number of genera listed for which we have doubts.

Tribe	Positive	No reports	Negative
Caesalpinieae	9 (1?)	22	17 (3?)
Cassieae	1	12	7 (1?)
Cercideae	0	3	2
Detarieae	8 (5?)	31	16 (3?)
Amherstieae	1	16	8 (1?)

but only in certain soils. There are several negative reports on this genus and others in the tribe (Allen & Allen, 1981; Faria et al., 1984; 1987a). It has been the subject of discussion taxonomically, having been placed variously in the Caesalpinieae, Amherstieae, and Detarieae (see Polhill & Raven, 1981). *Tamarindus indica* has been examined many times and found not to be nodulated (see Allen & Allen, 1981), but a recent paper (Ding et al., 1986) described nodules and nitrogenase activity.

Nodulation and Mycorrhizas

The rarity of nodulation in the Amherstieae is consistent with it being a uniform tribe, as is apparent also from its ectomycorrhizal nature (Alexander, this volume). Reports available for the Detarieae which may be nodulated indicate that they also form vesicular-arbuscular (VA) mycorrhizas (Table 7). Some genera with both positive and negative reports on nodulation *(Afzelia, Eperua, Intsia),* have both VA and ectotrophic mycorrhizas, not necessarily in the same species. It would be interesting to examine in more detail the association between type of mycorrhiza and nodulation (and also whether some doubtful reports of nodules are, in fact, ectotrophic mycorrhizas). The Caesalpinieae appear to be generally VA mycorrhizal, and this includes nodulating and non-nodulating genera (see Alexander, this volume, and Table 6A, B). Thus nodules appear to be associated with some of those genera that have VA mycorrhizas, while ectomycorrhizas and nodules appear to be mutually exclusive (see also Corby et al., 1983; Lamont, 1982). The exception so far is *Afzelia quanzensis,* which is both ectomycorrhizal and nodulated (Högberg & Nylund, 1981). McGee (1986) reported one species each from the Mimosoideae and Papilionoideae which had both VA and ectomycorrhizas.

Nodule structure

In all cases, structure of the infected cells is similar to that of the "aberrant" nodulators of the Dalbergieae *(Andira* and *Hymenolobium),* Millettieae *(Cyclolobium, Dahlstedtia),* and Robinieae *(Poecilanthe),* (Faria et al., 1987b) in that rhizobia within the nitrogen-fixing cells are confined by host cell wall material, resembling the infection threads of temperate legumes (Fig. 10). We are tentatively calling these structures persistent infection threads (Figs. 12, 14, 15). Such structures have previously only been reported from *Parasponia* (Ulmaceae), the only known non-legume to form nodules with *Rhizobium* (Trinick, 1979). We have not found them so far in nodules from the tribe Swartzieae, which is thought to be a bridge between the Caesalpinioideae and the Papilionoideae (Polhill & Raven, 1981). These and other studies in our laboratory have led us to formulate a framework for a possible evolutionary pathway for legume root nodules.

TABLE 6. Nodulation status of the Caesalpinieae. All references, except those listed, may be found in Allen & Allen (1981). Only definite reports are included; for all nonlisted genera no information has been found.

A. Positive reports Genus	Species	Reference or comment
Sclerolobium	*micropetalum*	
	grandiflorum	
	rugosum	Faria et al., 1987a
	pilgerianum	Faria et al., 1987a
Tachigali	*multijuga*	Faria et al., 1984
	paniculata var. *alba*	Faria et al., 1987a
Melanoxylon	*brauna*	Faria et al., 1984
Campsiandra	sp.	F. M. M. Magalhaes, pers. comm.[1]
Moldenhauera	*floribunda*	Faria et al., 1984
Colvillea	*racemosa*	1903 report, doubtful
Cordeauxia	*edulis*	needs confirmation
Erythrophleum	*atricanum*	
	suaveaolens	
Dimorphandra	*davisii*	
	exaltata	Faria et al., 1984
	mollis	Faria et al., 1984
	wilsonii	Faria et al., 1984
	vernicosa	Faria et al., 1986a
B. Negative Reports		
Gymnocladus	*dioica*	
Gleditsia	spp.	some very doubtful reports of nodules
Acrocarpus	*fraxinifolius*	Faria et al., 1987a
Peltophorum	*dubium*	Faria et al., 1987a
Schizolobium	*parahybum*	
	amazonicum	Faria et al., 1986
Delonix	*elata*	
	regia	sparse white nodules, presumably ineffective, have been reported
Cenostigma	*gardinerianum*	Faria et al., 1986
	tocantinum	Faria et al., 1986
Caesalpinia		13 species listed in Allen & Allen and a further five in Faria et al., 1984, 1987a[2]
Pterolobium	*stellatum*	
Hoffmannseggia	*densiflora*	
	microphylla	
	falcaria	
	burchellii	
Haematoxylum	*campechianum*	
Poeppigia	*procera*	Faria et al., 1984
Pterogyne	*nitens*	
Mora	spp.	swellings on some roots appear to be ectotrophic mycorrhizas, not nodules
Burkia	*africana*	

[1]Structure of these nodules has been studied by us.

[2]Ding et al. (1986) found nodules on *C. sappan*, but this has yet to be confirmed and contrasts with negative data in Allen & Allen (1981).

TABLE 7. Nodulation in the Detarieae. References may be found in Allen & Allen (1981) except where specified. Mycorrhizal status given as VA for vesicular-arbuscular or EM for ectomycorrhizas. Mycorrhizal data from Alexander (this volume): these apply to the genus only, not necessarily the species.

Genus	Species	Reference	Mycorrhiza	Comment
A. Positive reports				
Maniltoa	*grandiflora*		VA	one negative report also
Lysidice	*rhodostegia*		?	
Saraca	*declinata*		VA	an unidentified species was reported nodulated, but four species found not to nodulate
Afzelia	*quanzensis*	Högberg & Nylund, 1981	VA, EM	nodules found in Tanzania, but not Zimbabwe. *Afzelia africana* not nodulated
Intsia	*bijuga*		VA, EM	*Intsia bijuga* not always nodulated, negative reports on two other species
Hymenaea	*courbaril*	Faria et al., 1984	VA	not always nodulated two species not nodulated
Gossweilerodendron	*balsamiferum*		VA	one report only
Hardwickia	*pinnata*		?	one report (1903)
Phyllocarpus	*riedlii*		?	one report
Brownea	*ariza*	Faria et al., 1987a	?	*longipedicelata* not nodulated
Copaifera	*officinalis*	Faria et al., 1984	VA	very doubtful two species lacked nodules
Eperua	*falcata*		VA, EM	nodulation variable
B. Negative Reports				
Cynometra	*hankei*		VA	
	ramiflora			
	cauliflora			
Scorodophleus	*zenkeri*		VA	
Schotia	*brachypetala*		?	
	capitata			
	latifolia			
Peltogyne	*venosa*		VA	
	angustiflora	Faria et al., 1984		
	confertiflora	Faria et al., 1984		
Crudia	*parivoa*		?	
Guiboutia	*coleosperma*		VA	
	conjugata			
Colophospermum	*mopane*		VA	
Goniorrhachis	*marginata*		?	
Baikiaea	*plurijuga*		VA	
	insignis			
Sindora	*supa*	Faria et al., 1986	?	
	conchinensis			
Elizabetha	*paraensis*	Faria et al., 1986	?	
	princips			
	durissimma	Faria et al., 1986		
	speciosa	Faria et al., 1986		

TABLE 8. Legumes that do not produce typical root hairs. Data from this study except where specified.

Subfamily Genus	Species	Nodules	Reference or comment
Papilionoideae			
Arachis	*hypogaea*	+	Chandler, 1978
Stylosanthes	spp.	+	Chandler et al., 1981
Lonchocarpus	*muehlbergianus*	+	occasional hairs
Mimosoideae			
Parkia	*gigantocarpa*	−	African species nodulate
	pendula	−	Brazilian ones do not
	decussata	−	
Pithecellobium	*tortum*	+	other species have a few hairs
Enterolobium	*contortisiliquum*	+	
Acacia	*pulchella*	+	Barrow, 1977;
	raddiana	+	several species do
	auriculiformis	+	form hairs
	arora	+	
Mimosa	*scabrella*	+	occasional hairs seen
Neptunia	*oleracea*	+	occasional hairs seen
Caesalpinioideae			
Caesalpinia	*ferrea*	−	
	gisella	−	
	separia	−	
Cassia	*fistula*	−	some species form hairs
	javanica		
Schizolobium	*parahyba*	−	

IV. Evolution of Legume Nodules: A Hypothesis

Primitive legumes may not have had root hairs; many extant species still lack them (Lamont, 1982, table 8: see also comments by Corby in discussion following Sprent et al., 1987). *Rhizobium* and *Agrobacterium* may share a common ancestor (Sprent & Raven, 1985), which is likely to have entered plants via wounds. Thus we support the proposal (Sprent & Raven, 1985) that primitive nodules were formed not following root hair infections, but after bacteria entered breaks in the surface, such as those made by the emergence of lateral roots, i.e., as in the extant symbioses of *Arachis* and *Stylosanthes* (Table 1). From this beginning, two diverging systems evolved, with and without infection threads. In the latter, the sequence is similar to that currently found in *Arachis;* the most characteristic feature of this type of nodule, apart from the lack of infection threads, is the absence of interstitial cells (Fig. 6). This may impose functional limitations, since interstitial cells have a special role in at least the ureide-exporting legumes (Newcomb & Tandon, 1981). An advantage of the aeschynomenoid type of nodule is that it can, in theory, develop wherever entry can be gained and where meristematic potential exists. This is clearly seen in the formation of new nodules on old roots of *Dalbergia* sp. (Fig. 5).

The next development we perceive is infection between epidermal cells—such as now occurs in *Parasponia* (Lancelle & Torrey, 1984). Initial spread of rhizobia is intercellular, both through air spaces and by breakdown of the middle lamellae. This breakdown may extend to local areas of primary cell wall so that rapidly dividing bacteria push into the host cell, although never penetrate the plasmalemma (Fig. 3). The plant responds by secreting cell wall material and an infection-thread-like structure is formed (Figs. 12, 14, 15). This may develop extensively inside a single host cell, which is stimulated to enlarge. Conditions in such a cell allow derepression of the bacterial nitrogenase, and nitrogen fixation begins. Each cell of the active nitrogen-fixing region is separately

TABLE 9. Relations between root hairs and infection threads in nodulated legumes of the subfamilies Caesalpinioideae (C), Mimosoideae (M), and Papilionoideae (P). Characters found in the non-legume *Parasponia* (Pa) are included for comparison.

Character	C	M	P	Pa
Infection threads seen in				
(a) root cells	−	+/−	+/−	−
(b) young nodule cells	−	+	+/−	+
Persistent infection threads	+	−	+/−	+
Root hairs present	+/−	+/−	+/−	+
Hair infection, when hairs present	?/−	+/?	+/−	−

infected in this way: none subsequently divides and infection threads appear not to pass from cell to cell. A variable number of cells remains uninfected, forming a population of interstitial cells and allowing metabolic differentiation. Details of the origin of cell divisions which gave rise to such a nodule have not yet been examined, but since all such extant nodules of this type which have been examined are indeterminate (Fig. 4), we envisage intercellular passage of rhizobia in the wake of nodule meristematic activity. Some of the daughter cells would become infected as described above.

At some stage during evolution, infection threads began to cross from cell to cell (as seems to happen in *Parasponia*; Lancelle & Torrey, 1985), and is retained in advanced species such as *Galega* (Fig. 10). This could have started if some of the newly infected cells (described above) retained a limited capacity for division. Once infection threads passed across as well as between cells, products of the nodule apical meristem could be infected by branches of these threads. Soon after this, bacterial differentiation evolved, possibly in the manner suggested by Verma & Nadler (1984). These workers proposed that rhizobial walls become modified during nodule development and no longer evoke the hypersensitive reaction which leads to the secretion of host cell wall material around the bacteria. The latter are, however, retained within host membrane vesicles during their nitrogen-fixing period. At least two patterns seem to have emerged from such a system, in which the infected host cell does or does not retain the ability to divide. The familiar nodules of advanced herbaceous legumes, such as *Glycine max* (determinate) and *Trifolium* species (indeterminate), were formed. There is a distinct developmental pattern for each of the symbionts (Sprent, 1984; Sprent & Thomas, 1984). These particular forms do not concern us here.

A further corollary of the ability of infection threads to pass across and between cells is that the cortex of the newly infected root may be traversed in this way (see Fig. 10). Eventually infection threads would be formed at or near the surface of the root, and this immediately allows a root hair infection pathway (Fig. 1). Infection threads were a necessary prerequisite for root hair infection; the converse is not true. Thus even in extant legumes with root hairs, infections may occur through epidermal cells (as occasionally observed even in advanced species such as *Phaseolus lunatus;* Bal & Wong, 1982) as well as through lateral root junctions. This may be why some experienced investigators have searched in vain for infection threads in root hairs (e.g., Lawrie, 1983a, for *Aotus*). Extant legumes show many of the possible combinations of these features; see Table 9.

The advantages of root hair infection are a greater target area, earlier infection than is possible through lateral root junctions, and greater scope for complex recognition processes (Sprent & Raven, 1985). It may lend itself to exploitation to increase specificity and allow manipulation of particular host and bacterial genotypes (Sprent, 1986). On the other hand, extension of nodulating ability to non-nodulating legume genera (as opposed to non-nodulating genotypes of normally nodulating species) and to non-legume genera (as has occurred naturally in *Parasponia*) may be more likely through a simpler infection route. We might expect such infections, if they reach the interior of host cells, to be retained within host cell wall material, as in *Parasponia* and primitive

extant legume nodules. Study of the latter has relevance not only to legume evolution, but also to the genetic engineering of plants aimed at extending their nodulating capability.

V. Taxonomic Framework for the Data and Hypothesis

We agree with Lamont (1982) that root hairs may have evolved to cope with adverse conditions, including drought. We have evidence that root hair production is a much more plastic phenomenon than is often supposed. Salt (NaCl) stress, for example, can cause the normally bald epidermis of *Arachis hypogaea* to produce epidermal hairs (Sprent & McInroy, 1984). This suggests that the genetic information for root hairs may be present in a repressed state in some legumes. Thus perhaps it is not surprising to find hairy and hairless species within single genera and scattered among legume subfamilies (Table 8). We do not know if all the hairless species could produce hairs, given the correct stimulus. This complicates producing a taxonomic framework for the above hypothesis.

We shall assume that extant legumes were derived from a common hairless ancestor and that an ancestral *Rhizobium* gained entry to them in some way, probably initially through points of emergence of lateral roots. Modern aeschynomenoid nodules continued with simple infection processes and achieved a compatibility with the microsymbiont (release of rhizobia into membrane-bound vesicles) without going through a defined cell wall bounded phase. This is not inconsistent with the Aeschynomeneae being a fairly advanced tribe, but it requires a separate developmental line as Corby (1981) implied.

All the mimosoid nodules for which we have evidence show indeterminate growth (Fig. 4) in which infection threads carry rhizobia into newly formed cells. Few of those which have root hairs (e.g., *Prosopis*) have been shown to be infected in this way. On nodule characters, therefore, the Mimosoideae are fairly uniform, although there are some anomalies such as the variable nodulation of *Parkia* species. As an ancestral form for the Mimosoideae, *Dimorphandra* (Polhill et al., 1981; Crepet & Taylor, 1986) does not appear likely on nodule characters, since extant species of this genus have persistent infection threads; we have so far found none of these in mimosoid nodules, although many genera have now been examined (Faria et al., 1987b). On the other hand, *Dimorphandra* and *Sclerolobium* have very similar nodules and appear well-suited to be ancestors not only of other caesalpinioid nodules, but also many papilionoid forms. In considering this point, Polhill et al. (1981) linked the *Sclerolobium* group to the Sophoreae and hence other papilionoid tribes. We suspect that this will prove to fit in with nodule characters when we have examined more genera from the Sophoreae. The Swartzieae, which have many similarities with the Caesalpinioideae and are thought to be on the borderline between them and the Papilionoideae, have so far yielded no specimens with persistent infection threads (three species of *Swartzia* have been examined).

Polhill (1981b) suggested that the genera *Dipteryx*, *Taralea*, and *Pterodon* be grouped in a new tribe, the Dipterygeae. There are negative reports on nodulation in all of these genera (Allen & Allen, 1981; Faria et al., 1984, 1987a). This agrees with their grouping into a separate tribe and shows the tribe to be unusual (probably unique) among the Papilionoideae. Perhaps it is a separate line from the base caesalpinioid grouping.

Extant rhizobia (this name is used here in a general sense to include also those organisms now classified as *Bradyrhizobium*) vary greatly; some are more closely related to *Agrobacterium* than to other rhizobia (see references in Sprent & Raven, 1985). This suggests that there may have been more than one ancestral event leading to nodulation and/or considerable co-evolution of host and symbiont. As with the host plant part of the symbiosis, there is very little information on rhizobia from woody legumes; what there is suggests that a considerable reassessment of rhizobial biology may be necessary (e.g., Lawrie, 1983b).

With all these gaps in our knowledge, it is with some trepidation that we have acceded to an editor's (CHS) suggestion that we try to fit our observations into a general framework; Figure 16 is the outcome.

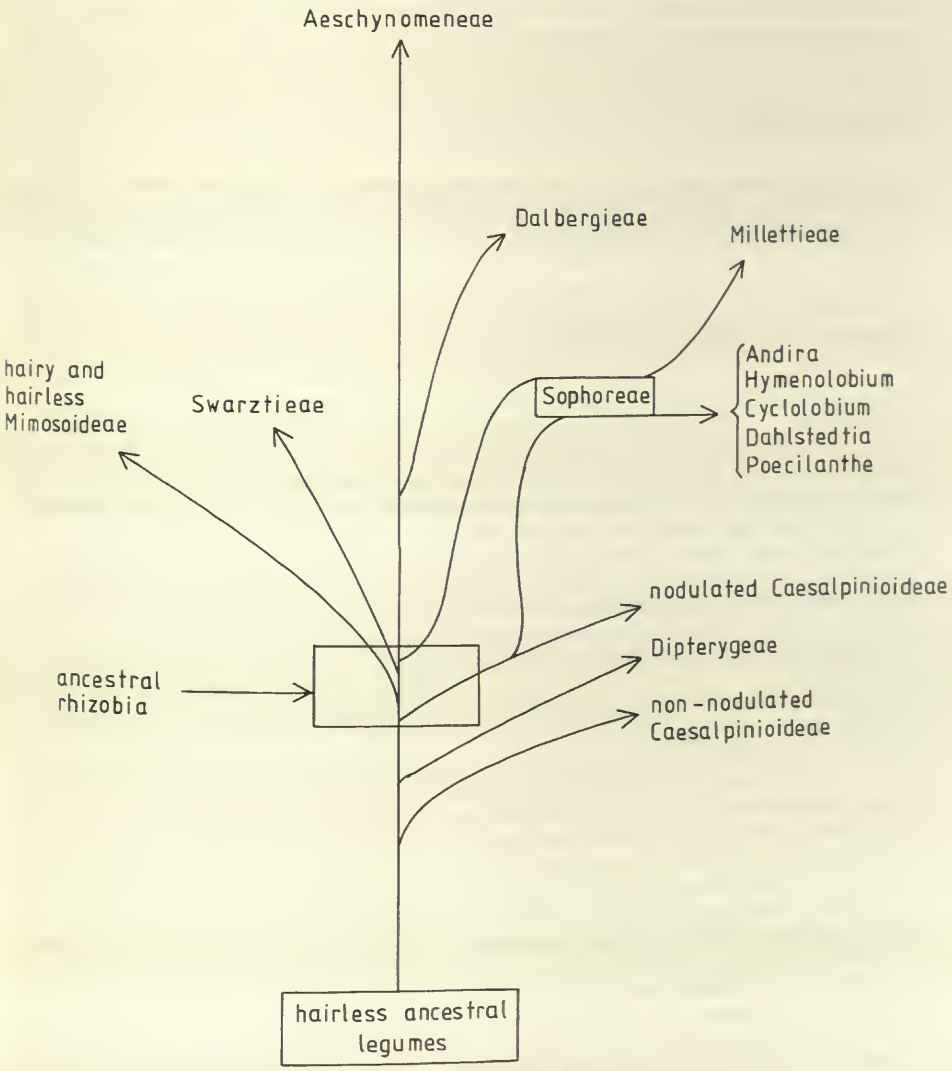


FIGURE 16. Possible relations between woody legumes, based on nodule characters.

ADDENDUM

In the three years since this manuscript was written we have obtained much new relevant information, some of which has led us to modify our views. Readers are particularly referred to:

Faria, S. M. de, G. Hay & J. I. Sprent. 1988. Entry of rhizobia into roots of *Mimosa scabrella* occurs between epidermal cells. J. Gen. Microbiol. 134: 2291–2296.

Sprent, J. I. & S. M. de Faria. 1988. Mechanisms of infection of plants by nitrogen fixing microorganisms. Pl. & Soil 110: 157–165.

Sprent, J. I. 1989. Which steps are essential to the formation of functional legume nodules? New Phytol. 111: 129–153.

Faria, S. M. de, G. P. Lewis, J. I. Sprent & J. M. Sutherland. 1989. Occurrence of nodulation in the Leguminosae. New Phytol. 111: 607–619.

Acknowledgments

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References

Allen, O. N. & E. K. Allen. 1981. The Leguminosae. Macmillan, London.

Bal, A. K. & P. P. Wong. 1982. Infection processes and sloughing off of rhizobial outer membrane in effective nodules of lima bean. Canad. J. Microbiol. 28: 890–896.

Baretta-Kuipers, T. 1981. Wood anatomy of Leguminosae: its relevance to taxonomy. Pp. 677–705 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Barrow, N. J. 1977. Phosphorus uptake and utilization by tree seedlings. Austral. J. Bot. 25: 571–584.

Bauer, W. D. 1981. Infection of legumes by Rhizobia. Annual Rev. Pl. Physiol. 32: 407–449.

Chandler, M. R. 1978. Some observations on infection of *Arachis hypogaea* L. by *Rhizobium*. J. Exp. Bot. 29: 749–755.

———, R. A. Date & R. J. Roughley. 1982. Infection and root-nodule development in *Stylosanthese* species by *Rhizobium*. J. Exp. Bot. 33: 47–57.

Corby, H. D. L. 1974. Systematic implications of nodulation among Rhodesian legumes. Kirkia 9: 301–329.

———. 1981. The Systematic Value of Leguminous Root Nodules. Pp. 657–669 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———, R. M. Polhill & J. I. Sprent. 1983. Taxonomy. Pp. 1–35 *in* W. J. Broughton (editor), Nitrogen Fixation Volume 3: Legumes. Clarendon Press, Oxford.

Crepet, W. L. & D. W. Taylor. 1986. Primitive mimosoid flowers from the paleocene-eocene and their systematic and evolutionary implications. Amer. J. Bot. 73: 548–563.

Ding, M- M., W. M. Xi & L- Y. Liao. 1986. A survey of the N_2ase activities of nodules of tree legumes, including *Tamarindus indica* a species not widely known to nodulate, in artificial forests in Dainbai, Guangdong, China. Nitrogen Fixing Tree Reports 4: 9–10.

Duhoux, E. 1984. Ontogenese des nodules caulinaires du *Sebania rostrata* (legumineuses). Canad. J. Bot. 62: 982–994.

Faria, S. M. de, A. A. Franco, R. M. de Jesus, M. de S. Menandro, J. B. Baitello, E. S. F. Mucci, J. Dobereiner & J. I. Sprent. 1984. New nodulating legume trees from South-east Brazil. New Phytol. 98: 317–327.

———, H. C. de Lima & E. F. C. Campelo. 1985. A morfologia dos nodulos radiculares na taxonomia da tribo Dalbergieae. Anais XXXVI Congresso de Botanica. P. 370. Sociedade Botânico do Brasil, Curitiba PR.

———, ———, A. A. Franco, E. S. F. Mucci & J. I. Sprent. 1987a. Nodulation of legume trees from South East Brazil. Pl. & Soil 99: 347–356.

———, S. McInroy & J. I. Sprent. 1987b. The occurrence of infected cells, with persistent infection threads, in legume root modules. Canad. J. Bot. 65: 533–538.

———, J. M. Sutherland & J. I. Sprent. 1986. A new type of infected cell in root nodules of *Andira* spp. (Leguminosae). Pl. Sci. 45: 143–157.

Fraser, L. 1931. The reaction of *Viminaria denudata* to increased water content of the soil. Proc. Linn. Soc. New South Wales 56: 391–406.

Geesink, R. 1981. Tephrosieae. Pp. 245–260 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

———. 1984. Scala Millettiearum. Leiden Botanical Series. E. J. Brill, Leiden University Press. 8: 133.

Goldblatt, P. 1981. Cytology and the Phylogeny of Leguminosae. Pp. 427–463 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Harris, J. O., E. K. Allen & O. N. Allen. 1949. Morphological development of nodules on *Sesbania grandiflora* Poir. with reference to the origin of nodule rootlets. Amer. J. Bot. 36: 651–661.

Högberg, P. & J. E. Nylund. 1981. Ectomycorrhizae in coastal miombo woodland of Tanzania. Pl. & Soil 63: 283–289.

Irwin, H. S. & R. C. Barneby. 1982. American Cassanieae: synoptical revision of Leguminosae, tribe Cassinieae, sub-tribe Cassanieae in the New World. Mem. New York Bot. Gard. 35: 1–454.

Jimbo, T. 1927. Physiological anatomy of the root nodule of *Wistaria sinensis*. Proc. Imp. Acad. Japan 3: 164–166.

Lamont, B. 1982. Mechanisms for enhancing nutrient uptake in plants, with particular reference to Mediterranean South Africa and Western Australia. Bot. Rev. (Lancaster) 48: 597–689.

Lancelle, S. A. & J. G. Torrey. 1984. Early development of *Rhizobium*-induced root nodules of *Parasponia rigida*. 1. Infection and early nodule initiation. Protoplasma 123: 26–37.

——— & ———. 1985. Early development of *Rhizobium*-induced root nodules of *Parasponia rigida*. II. Nodule morphogenesis and symbiotic development. Canad. J. Bot. 63: 25–35.

Lavin, M. 1987. A cladistic analysis of the tribe Robinieae (Papilionoideae, Leguminosae). Pp. 31–64 *in* C. H. Stirton (editor), Advances in Legume Systematics, 3.

Lawrie, A. 1983a. Infection and nodule development in *Aotus ericoides* G. Don, a woody native Australian legume. J. Exp. Bot. 34: 1168–1180.

———. 1983b. Relationships among rhizobia from native Australian legumes. Appl. & Environ. Microbiol. 45: 1822–1828.

McGee, P. 1986. Mycorrhizal associations of plant species in a semiarid community. Austral. J. Bot. 34: 585–593.

Newcomb, E. H. & S. R. Tandon. 1981. Uninfected cells of soybean root nodules: ultrastructure suggests key role in ureide production. Science 212: 1394–1396.

Norris, D. O. 1959. The role of calcium and magnesium in the nutrition of *Rhizobium*. Austral. J. Agric. Res. 10: 651–698.

Olsson, J. E. & B. G. Rolfe. 1985. Stem and root nodulation of the tropical legume *Sesbania rostrata* by *Rhizobium* strains ORS-571 and WE7. J. Pl. Physiol. 121: 199–210.

Pankhurst, C. E. & W. T. Jones. 1978. Effectiveness of *Lotus* root nodules II. Relationship between root nodule effectiveness and 'in vitro' sensitivity of fast-growing *Lotus* rhizobia to flavolans. J. Exp. Bot. 30: 1085–1093.

Polhill, R. M. 1981a. Papilionoideae. Pp. 191–208 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

———. 1981b. Dipteryxeae. Pp. 231–232 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & P. H. Raven. 1981. Advances in Legume Systematics, Part 1 & 2. Royal Botanic Gardens, Kew.

———, ——— & C. H. Stirton. 1981. Evolution and Systematics of the Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & M. Sousa. 1981. Robinieae. Pp. 283–288 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Raven, J. A. 1986. Long distance transport of calcium. Pp. 241–255 *in* A. Trewavas (editor), Cellular Aspects of calcium in plant development. Plenum Press, New York.

Spratt, E. 1919. A comparative account of the root nodules of the Leguminosae. Ann. Bot. (London) 33: 189–199.

Sprent, J. I. 1980. Root nodule anatomy, type of export product and evolutionary origin in some Leguminosae. Plant, Cell and Environment 3: 35–43.

———. 1981. Functional evolution in some papilionoid root nodules. Pp. 671–676 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

———. 1984. Nitrogen fixation. Pp. 249–276 *in* M. B. Wilkins (editor), Advanced Plant Physiology. Pitman, London.

———. 1985. Nitrogen fixation in arid environments. Pp. 215–229 *in* G. E. Wickens, J. R. Gooden & D. V. Field (editors), Plants for Arid Lands. George Allen & Unwin, London.

———. 1986. Benefits of *Rhizobium* to agriculture. Trends in Biotechnology 4: 124–129.

_____ & S. McINROY. 1984. Effects of salinity on growth and nodulation of *Arachis hypogaea*. P. 546 *in* C. Veeger & W. E. Newton (editors), Advances in Nitrogen Fixation Research. Nijhof Pudoc, Wageningen.

_____ & J. A. RAVEN. 1985. Evolution of nitrogen fixing symbioses. Proc. Roy. Soc. Edinburgh 85B: 215–237.

_____, J. M. SUTHERLAND & S. M. DE FARIA. 1987. Some aspects of the biology of nitrogen fixing organisms. Phil. Trans. Roy. Soc. B: 317: 111–129.

_____ & R. J. THOMAS. 1984. Nitrogen nutrition of seedling grain legumes: some taxonomic, morphological and physiological constraints. Plant, Cell and Environment 7: 637–645.

SUTHERLAND, J. M. & J. I. SPRENT. 1984. Calcium oxalate crystals and crystal cells in determinate root nodules of legumes. Planta 161: 193–200.

_____ & _____. Nitrogen Fixation by Legume Trees. *In*: Use of Nuclear Techniques in Studying the Roles of Trees in Restoring and Maintaining Soil Fertility. Report of IEAE Workshop Vienna, (in press).

TRINICK, M. J. 1979. Structure of nitrogen-fixing root nodules formed by *Rhizobium* on roots of *Parasponia andersonii* Planch. Canad. J. Microbiol. 25: 565–578.

TSIEN, H. C., B. I. DREYFUS & E. L. SCHMIDT. 1983. Initial stages in the morphogenesis of nitrogen-fixing stem nodules of *Sesbania rostrata*. J. Bacteriol. 156: 888–897.

VAUGHN, K. C. & C. D. ELMORE. 1985. Ultrastructural characterization of nitrogen-fixing stem nodules on *Aeschynomene indica*. Cytobios 42: 49–62.

VERMA, D. P. S. & K. NADLER. 1984. Legume-*Rhizobium*-symbiosis: host's point of view. Pp. 57–93 *in* D. P. S. Verma & T. H. Hohn (editors), Genes in Microbe-plant Interactions. Springer, Wein.

WHITTON, B. A. 1962. Forests and dominant legumes of the Amatuk region, British Guiana. Caribbean Forest. 23: 35–57.

YATAZAWA, M. & M. SUSILO. 1980. Development of the upper stem nodules in *Aeschynomene indica* under experimental conditions. Soil Sci. Pl. Nutr. 26: 317–320.

_____, S. YOSHIDA & E. MAEDE. 1984. Fine structure of root nodule of *Aeschynomene indica* L. Soil Sci. Pl. Nutr. 30: 405–416.

ECOSYSTEM ESTIMATES OF N_2-FIXATION AND INVESTIGATIONS OF NODULE METABOLISM USING VARIATIONS IN THE NATURAL ABUNDANCE OF ^{15}N

Daniel H. Kohl[1] and Georgia Shearer[1]

ABSTRACT

Two uses of variation in the natural abundance of ^{15}N in legumes are described. 1) Estimating the contribution of atmospheric N_2 to the total nitrogen of legumes: Problems in applying conventional methods in field settings (especially in natural ecosystems) have led to tests of a method based on small differences in N isotope abundance between N_2-fixing and non-N_2-fixing plants. Advantages of and problems with this method are described. We conclude that in most circumstances the method is capable of realistic, although not highly precise, estimates. Application of the method to several desert ecosystems is described. 2) Nitrogen metabolism within nodules: The natural ^{15}N abundance of most plant parts is fairly uniform and close to that of their N source(s). The substantial enrichment of nodules of certain legumes is a striking exception. This enrichment apparently is related to N_2-fixation since only nodules that are actively fixing N_2 are enriched in ^{15}N, and most of the ^{15}N enrichment is in the bacteroids. Hypotheses so far tested do not account for the unique ^{15}N enrichment of nodules, but the observation is consistent with a hypothesis that the carbon skeletons needed to fuel N_2-fixation are imported from the host to the bacteroid as amino acids.

I. INTRODUCTION

This paper addresses two areas in which ^{15}N natural abundance variation data relate to issues in legume biology. The first area will be addressed in more detail than the second, although neither will be covered exhaustively. A fuller treatment of ecosystems estimates of N_2-fixation appears in Shearer & Kohl (1986). Discussion of nodule metabolism includes unpublished data and therefore must be considered to be a preliminary report.

Peter Felker, well known from his extensive work with *Prosopis* (Felker & Clark, 1980, 1982; Felker et al., 1981), first interested us in the possibility of collaborating on a study of N_2-fixation in warm desert ecosystems. He became interested in our work because the conventional methods for estimating the quantity of N fixed within an ecosystem are not very satisfactory. These conventional methods include the acetylene reduction assay, the N difference method, and methods involving adding ^{15}N enriched material to the soil. Each of these methods poses significant problems for the ecosystem scientist. We review them briefly below.

II. CONVENTIONAL METHODS OF ESTIMATING N_2-FIXATION

Figure 1 shows the acetylene reduction method in principle. (For a complete treatment of the method see, for example, Turner & Gibson, 1980.) It is the most widely used conventional method for estimating N_2-fixation. In this method, nodules are placed in an atmosphere of acetylene. The enzyme responsible for reducing N_2 to NH_3 accepts acetylene as a substrate and reduces it to ethylene. The units in which the results are reported are micromoles of ethylene produced per unit time per g nodule. The method is fast, very sensitive and inexpensive (assuming that a gas chromatographic instrument is available). But there are also disadvantages. Some of these are general,

[1]Department of Biology, Washington University, St. Louis, Missouri 63130, U.S.A.

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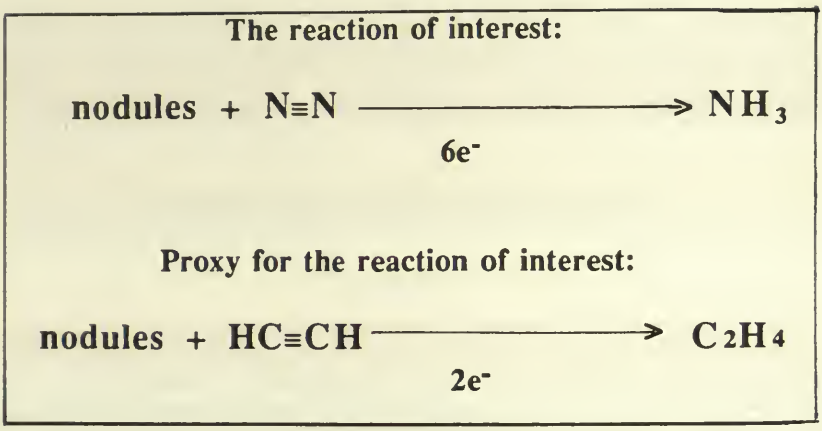

FIGURE 1. Acetylene Reduction Assay for Nitrogenase Activity. Nitrogen-fixation involves the six electron reduction in the nodule of N_2 to NH_3. Nitrogenase in the nodule will also convert acetylene to ethylene in a two electron reduction. The ethylene is then measured gas chromatographically as an indication of the activity of the N_2-fixing enzyme system in the nodule. The results are reported as micromoles ethylene produced/unit time/mg nodule.

some specific to the ecosystems scientist. Problems include the following. i) It is necessary to convert the quantity of acetylene reduced to the quantity of N_2 reduced. Usually the quantity of acetylene reduced is divided by three, since the reduction of acetylene to ethylene requires two electrons while the reduction of N_2 to NH_3 requires six electrons. However, in practice, measured values of this ratio have varied between 1.5:1 and 25:1. In a recent intensive study in a *Eucalyptus marginata* (Myrtaceae) forest ecosystem in western Australia, Hansen et al. (1987) found values between 1.6 and 4.8. ii) Ecosystem scientists most often are interested in obtaining information about the amount of N_2 fixed in a growing season. Since the acetylene reduction method gives an instantaneous value of the measured parameter, it is necessary to integrate the values obtained over time. This is a nontrivial matter. Acetylene reducing activity depends dramatically on the phenological stage of the plant as well as the environmental conditions at any given phenological stage. For example, acetylene reducing activity is sensitive to rainfall (which reduces the soil aeration) and to even modest changes in light intensity, and it shows extreme diurnal variation. As a result, any attempt to quantify the amount of N fixed over a growing season requires many measurements. The problems of integrating over a growing season lead to the loss of much of the speed and precision that make the method attractive in settings where, for example, the required information is the relative N_2-fixing capabilities of two cultivars growing side by side in a greenhouse. iii) Ecosystems scientists are more likely to need information about the quantity of N fixed on a per hectare basis than about the N_2-fixing ability of a small group of root nodules. In order to translate acetylene reduction rates to a per hectare basis, it is first necessary to estimate the quantity of nodules in a hectare, since the measurement is made on a sample of nodules. This is often extremely difficult, especially when the ecosystem is dominated by large, woody plants. As an example, consider *Prosopis glandulosa* (honey mesquite). Its roots often descend to the capillary fringe of the water table, many meters deep in the soil profile. Greenhouse studies demonstrate that effective nodules form in the capillary fringe (Felker & Clark, 1982). Finding nodules in the field on the roots of this large, woody, phreatophytic legume proved so elusive that many doubted it fixed N_2. It is impossible to estimate the quantity of nodules in a hectare under such circumstances. In fact, in this case, it is usually impossible to find nodules to use in the acetylene reduction assay.

Nitrogen balance studies are a second conventional means of estimating N_2-fixation. The prin-

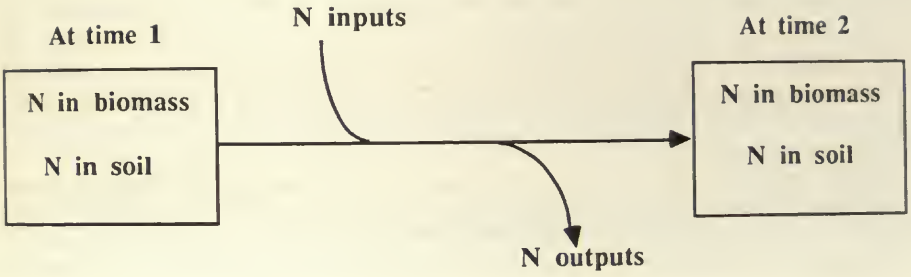

FIGURE 2. The Nitrogen Balance Method of estimating N_2-fixation. The N in biomass and soil N is measured at time 1 and time 2. All of the N inputs and outputs, except N_2-fixation, are measured during the interval between time 1 and time 2. The quantity of N_2-fixation can then be calculated by difference.

ciple of the method is shown in Figure 2. If the N in the soil and biomass is known at the beginning and end of the experiment, if all the outputs of N from the system are measured, and if all the inputs into the system except N_2-fixation are measured, then the quantity of N_2 fixed can be calculated by difference. This approach requires a massive effort. (The ongoing work at Hubbard Brook, New Hampshire, U.S.A. is an outstanding example of the application of the N balance method, and it illustrates the scale of the effort necessary to produce interesting results. See for example, Bormann et al., 1977). The pool sizes and the fluxes, which are required as the raw data of the calculation, usually cannot be measured with great accuracy. Therefore, errors in the estimates of the parameter values often result in large uncertainties in the calculated value of the quantity of N_2 fixed. Finally, there is no good way to measure some of the inputs and outputs. For example, the amount of N lost from the system via denitrification must be estimated, since there are no adequate methods for measuring it in most cases.

A third more or less conventional method for measuring N_2-fixation in field settings involves adding ^{15}N labeled inorganic fertilizer to the soil. The principle of the method is illustrated in Figure 3. Addition of ^{15}N enriched fertilizer results in establishing two sources of N, whose ^{15}N abundances differ, to the N_2-fixing plant. One source is atmospheric N_2 and the other is the soil N pool. The latter contains N mineralized from soil (whose ^{15}N abundance is usually slightly greater than that of atmospheric N_2) and the added N (whose ^{15}N abundance is much greater than that of atmospheric N_2). One proceeds as follows: 1) Establish the ^{15}N abundance of the "soil and fertilizer N pole." This is where the largest errors enter. 2) Assign the ^{15}N abundance of atmospheric N_2 to the lower pole. 3) Measure the ^{15}N abundance of the N_2-fixing plant. 4) Interpolate between the poles to calculate the %N derived from air (%Ndfa).

How is the ^{15}N abundance of the "soil and fertilizer N pole" evaluated? Given the heterogeneity of the system, direct measure of that pool would have an unacceptably large standard error. Instead, it is noted that non-N_2-fixing reference plants sample this pool as their only source of N, integrating the ^{15}N abundance of the pool as they grow. The ^{15}N abundance of these plants is taken to be a reflection of the ^{15}N abundance of the "soil and fertilizer N pool" available to the N_2-fixing plant. Thus, the central assumption is that the N_2-fixing and the reference plants take soil N and fertilizer N in the same proportion. That is, one assumes that the ^{15}N taken up from the "soil and fertilizer N pool" by the N_2-fixing and the reference plants have identical ^{15}N abundances. An advantage of this method is that the poles of the interpolation are far apart so the measurement error is small.

There are two main disadvantages of the method described above. 1) Added inorganic N perturbs the system. Inorganic N is a known inhibitor of N_2-fixation. In addition, the added fertilizer N may act as starter N and result in a more extensive root system earlier in the plant's development. 2) The overwhelming disadvantage of the method is that the basic assumption is seldom valid in natural ecosystems. This is illustrated by the data in Figure 4. In this experiment, Witty (1983) added ^{15}N

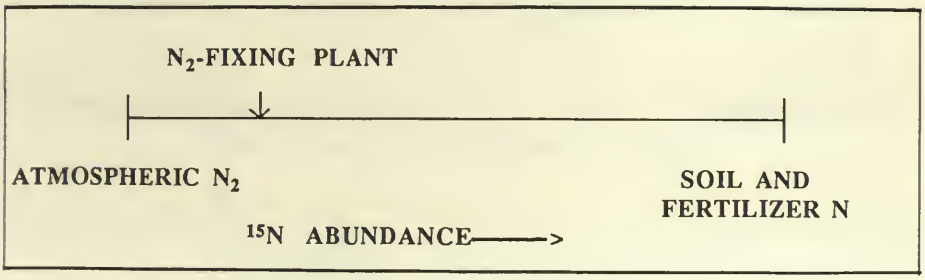

FIGURE 3. The Isotope Dilution Method for estimating the quantity of N_2-fixation. ^{15}N labeled fertilizer is added to the soil to increase ^{15}N the abundance of the "soil and fertilizer N" pole. Non-N_2-fixing reference plants and the N_2-fixing plant of interest are assumed to take N of the same ^{15}N abundance from the soil/fertilizer pool. Nitrogen in the N_2-fixing plant comes in part from the soil/fertilizer N pool, and from atmospheric N_2. If the ^{15}N abundance of atmospheric N_2, of the soil/fertilizer N pool, and of the tissue of the N_2-fixing plant of interest are known, then the % of N in the N_2-fixing plant which originated as atmospheric N_2 can be calculated by interpolating between the two indicated poles—namely, the "atmospheric N_2" and the "soil and fertilizer N" pools.

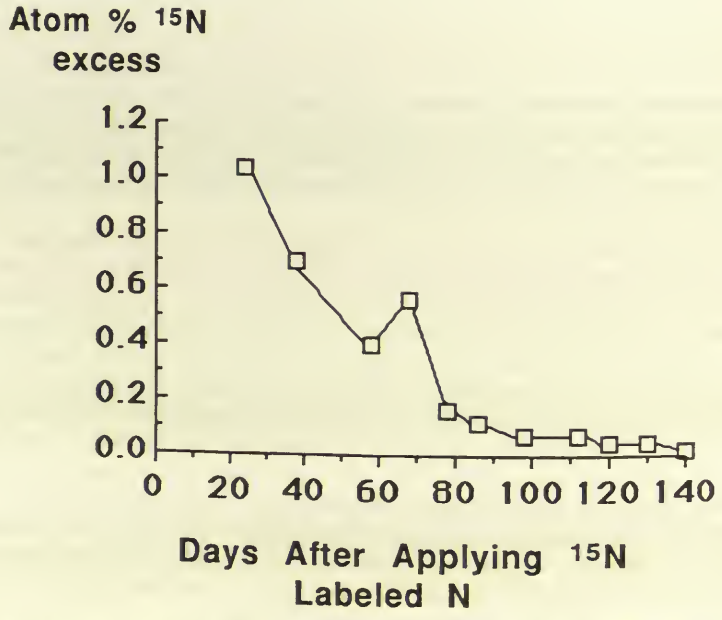

FIGURE 4. The isotopic enrichment of KCl extracted soil N beneath grass receiving 30 kg N/ha at 3.865 atom % ^{15}N excess. Modified from Witty (1983).

enriched material to the soil and recorded its dilution by inorganic N mineralized from soil organic matter. The ^{15}N abundance of the inorganic N in the soil system (the N that presumably would be available to the plant) changed dramatically with time since N with an ^{15}N abundance close to that of atmospheric N_2 entered the pool through the breakdown of soil organic matter. This result poses problems for the method under consideration. For example, suppose that the N_2-fixing plant took up N from the soil pool earlier than the reference plant did. In that case, the central assumption—

that the N taken up from the "soil and fertilizer N" pool by the N_2-fixing and the reference plants has identical ^{15}N abundance—would be clearly incorrect. The result would be that the interpolation dramatically understates the quantity of N fixed. (For discussion of such a case, see Shearer & Kohl, 1986.) If the N_2-fixing plant had taken up N later than the reference plant did, the result would have been to dramatically overstate the quantity of N_2 fixed. A less widely used variation of this method overcomes this difficulty and is suitable in some circumstances (Kohl & Shearer, 1981). In this method, material artificially enriched in ^{15}N is added to the soil prior to the start of the experiment and is allowed to become incorporated into the soil organic matter. This reduces the time dependence of the ^{15}N abundance of plant-available soil N. However, a satisfactory degree of incorporation might take six months to two years and, for this and other reasons, is more feasible for greenhouse experiments than for ecosystem field studies.

In summary, our view is that the more conventional methods for estimating N_2-fixation are not well suited to the needs of the ecosystems scientist. That is the reason Felker, who was interested in the role of N_2-fixation by large, woody legumes, was seeking a new approach. Felker thought the method for estimating N_2-fixation that we had been working on might be useful in field settings with large, woody plants. This method involves using small differences in the natural abundance of the heavy stable isotope of N, ^{15}N, to determine the relative contribution of atmospheric N_2 versus soil N to a nitrogen-fixing plant.

III. Basis of the Natural ^{15}N Abundance Method of Estimating N_2-Fixation

Before describing the principle of the method, we digress to introduce the units used in this work. $\delta^{15}N$ is the quantitative measure of ^{15}N abundance. $\delta^{15}N$ is defined as:

$$\delta^{15}N = \{[R_{spl}-R_{std}]/R_{std}\}\ 1000‰$$

where "spl" stands for sample, "std" stands for standard and R is the fraction of the molecules containing an ^{15}N atom. That is,

$$R = {}^{15}N/[{}^{15}N + {}^{14}N].$$

For atmospheric N_2,

$$R = 0.003663.$$

That is, 0.3663% of the N atoms in the atmosphere are the heavier isotope, ^{15}N. All of the rest are ^{14}N. Atmospheric N_2 is the ultimate reference standard. A laboratory working standard, either tank N_2 or N_2 generated from $(NH_4^+)_2SO_4$, is often used. This laboratory standard is then measured with respect to atmospheric N_2. Thus, if a sample has 1% more ^{15}N in it than does atmosphere (that is, if the sample has 0.0037 atom % excess), its $\delta^{15}N$ value will be +10‰. Likewise, a value of 0.00037 atom % excess corresponds to $\delta^{15}N = +1‰$. The range of variation commonly observed in the N cycle is:

$$-10‰ < \delta^{15}N < +20‰.$$

By contrast, when ^{15}N enriched material is used as a tracer, 1 atom % excess (i.e., 1.3663 atom % ^{15}N) would be a very modest level of enrichment. This corresponds to +2730‰ in $\delta^{15}N$ units. The natural abundance variations of a few ‰ which occur in the N cycle can be routinely measured if care is taken and the appropriate instrument is used.

The principle of the ^{15}N natural abundance method is illustrated in Figure 5. Just as in the isotope dilution method, the ^{15}N abundances of two poles are established, the ^{15}N abundance of the plant is measured, and the percent N derived from atmospheric N_2 (%Ndfa) is calculated by interpolating between the two poles. The pole labeled "biologically fixed N" is the ^{15}N abundance of atmospheric N_2 after it has been incorporated into plant tissue. (We often call this pole the "atmospheric

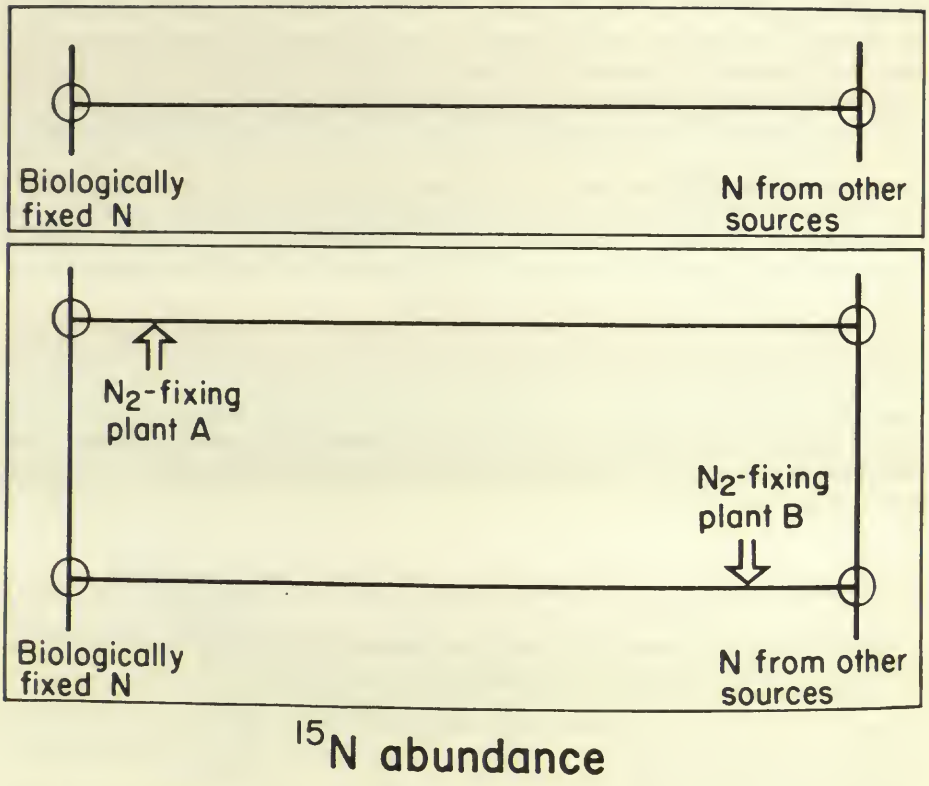

FIGURE 5. The $\delta^{15}N$ Natural Abundance Method of estimating N_2-fixation. Most of the N of Plant A (about 90%) is derived from atmospheric N_2. Most of the N of Plant B is derived from sources other than atmospheric N_2. (Only about 15% is derived from atmospheric N_2.)

N_2'' pole.) The pole labeled ''N from other sources'' is mostly inorganic N mineralized from soil and subsequently incorporated into plant tissue. (We often call this pole the ''soil N'' pole.) The idea is simple. The ^{15}N abundance of N_2-fixing plant A is measured. Its ^{15}N abundance is close to that of biologically fixed N. The researcher then concludes that most of the N in that plant originated as atmospheric N_2. The ^{15}N abundance of Plant B is measured. It is very close to the ^{15}N abundance of the ''N from other sources'' pole. The researcher concludes that, while this plant species may be capable of fixing N_2, in this setting this specific plant is not taking much of its N from the atmosphere. If the data seem to warrant a more quantitative measure, the researcher calculates the %Ndfa by interpolating between the two poles. This method has several advantages. 1) It gives an integrated estimate, as opposed to an estimate for a single point in time. 2) The tissue can be collected in the field and brought back to the lab. (Living tissue is not needed for the measurements.) 3) The ^{15}N abundance of the soil N pool is much more homogeneous in space and time than it is when ^{15}N enriched fertilizer is added to the soil N pool, as is done in the more conventional isotope dilution method discussed earlier.

There are also problems with the method. The poles of the interpolation are quite close together (see Table 2), thus considerable care must be taken in making the measurement. The method is expensive. Two good technicians can produce about 15–20 $\delta^{15}N$ measurements per day, including sample preparation. The distance between the poles will, of course, vary from site to site. The further the poles are separated from each other in ^{15}N abundance, the more accurate the estimate of

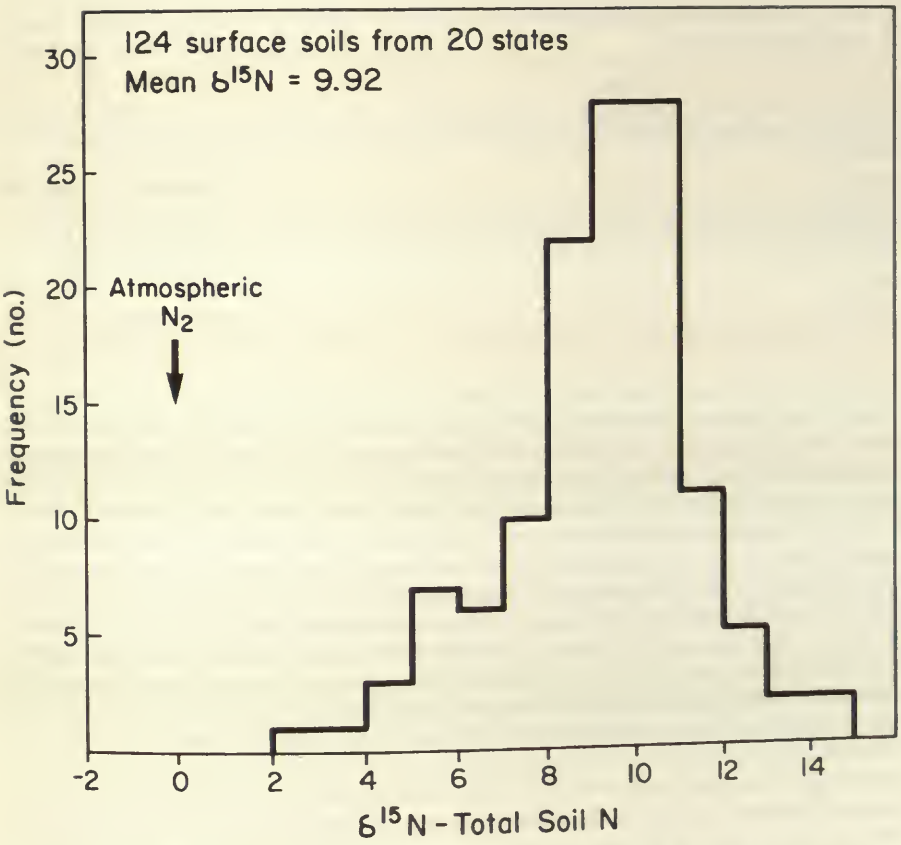

FIGURE 6. Frequency distribution of $\delta^{15}N$ values of total soil N. Samples were collected near the soil surface. Modified from Shearer et al. (1978).

%Ndfa for any given number of measurements will be. If the ^{15}N abundances of the poles are experimentally indistinguishable from each other, then the method cannot be used at that site. This sometimes happens.

How probable is it that the two poles of the interpolation have ^{15}N abundances that are measurably different from each other? Figure 6 presents the results of an extensive soil survey. The ^{15}N abundance of the total N from soil serves, in a crude way, as a proxy for the right-hand pole of the interpolation, the "N from other sources," or "soil N" pole. Surface soil samples from 124 sites in 20 states had higher $\delta^{15}N$ values than did atmospheric N_2—the mean value was about 9‰ higher than for atmospheric N_2. This is an easily measured difference. These data suggest that in many cases the two poles of the interpolation are far enough apart to allow the ^{15}N natural abundance method to work at most sites. However, it must be determined at each site whether N derived from soil has a measureably different ^{15}N abundance than does N derived from atmosphere. The method of establishing the poles of the interpolation is of considerable practical importance. The "biologically fixed N" pole is determined by bringing seeds from the plant of interest into the greenhouse and forcing the plant to live on atmospheric N_2 alone. That is, the plant is grown hydroponically in a mineral medium that contains no N. Thus, all of the N in that plant comes from the atmosphere (and the seed), and its ^{15}N abundance is an accurate reflection of the value of the "biologically fixed N" pole (after a correction is made for the seed N).

TABLE 1. ^{15}N abundance of soybean plants whose only source of N was atmospheric N_2. E = entire organism; A = aboveground plant parts.

Cultivar	Tissue measured	$\delta^{15}N$ ‰	Source
'Hodgson'	A	−1.6	Mariotti et al., 1980
'Hodgson'	A	−3.6	Domenach & Corman, 1984
'Amsoy'	A	−1.2	Mariotti et al., 1980
'Chippewa'	A	−1.5	Mariotti et al., 1980
'Wells'	A	−1.3	Mariotti et al., 1980
'Harosoy'	E	+1.0	Shearer et al., 1980
'Harosoy'	A	0	Shearer et al., 1980

Table 1 shows measurements made in a number of laboratories of the $\delta^{15}N$ of the "biologically fixed N" in soybeans. The main feature of the data is that the ^{15}N abundance of the plants is quite close to that of atmospheric N_2. There is little isotopic fractionation[2]. That is, the isotope abundance of atmospheric N_2 has not been altered much despite the fact that the N–N triple bond has been reduced to NH_3, as a result of N_2-fixation. The largest source of difference among the values is undoubtedly the error involved in translating the laboratory working standard to the absolute value of the ^{15}N abundance for atmospheric N_2. The latter involves a nontrivial measurement of the ^{15}N abundance of the working standard with respect to atmospheric N_2. Fortunately, an error of this type does not introduce error into estimates of the fractional contribution of N_2-fixation, since it results in an equal displacement of ^{15}N values of all three variables needed for the calculation—namely, $\delta^{15}N$ values of the "soil N" pole, the "fixed N" pole, and the N_2-fixing plant in the environment of interest. Another factor contributing to the differences seen in Table 1 is that the value is based on the entire plant in one case and on the aboveground parts only in the others.

Establishing the value of the "N from other sources" pole is more difficult than the "fixed N" pole, in principle and in practice. Usually, "other sources" means soil-derived N. The ^{15}N abundance of the total soil N only crudely approximates the N in the plant that is derived from soil N, since total soil N may lose its isotopic identity when organic N is first transformed to mineral N and then incorporated into plant tissues. Instead of using the ^{15}N abundance of total soil N for this pole, one samples non-N_2-fixing plants growing near the N_2-fixing plant of interest on the theory that both types of plants are taking N from a soil N pool of the same ^{15}N abundance. Since this assumption may not always be strictly valid, we try to collect four or five non-N_2-fixing reference plants for each N_2 fixer. The standard error of the mean of this group of reference plants gives an indication of the homogeneity of ^{15}N in the soil N pool. These issues (along with others, such as: What plant part should be collected? *and* At what point in the growing season should collections be made?) are treated in detail in Shearer & Kohl (1986).

The combined weight of all possible problems can be evaluated by comparing the results using the natural ^{15}N abundance method to results obtained by conventional methods when the conditions for the latter are ideal. This has been done on a number of occasions. The natural ^{15}N abundance method has been tested in greenhouse and field settings and has been shown to give reliable results under most conditions. (These tests are reviewed in some detail in Shearer & Kohl, 1986). The method cannot give estimates of %Ndfa that are good to within 1 or 2%. But in most cases it can

[2]Isotopic fractionation is the phrase used to describe the tendency of molecules containing the lighter isotope to react more quickly than those containing the heavier isotope (a few percent more quickly in the case of ^{14}N vs. ^{15}N). Thus, for the reaction A → B, the first increment of B made will be lighter than the starting material. Conservation of mass requires the weighted average of A and B to have the same ^{15}N abundance as the starting material. Thus, since B is lighter than the starting material, the unreacted A will be heavier than the starting material.

tell you whether N_2-fixation makes an important or insignificant input to an ecosystem, even an ecosystem dominated by large, woody species.

IV. Estimations of N_2-Fixation in Desert Ecosystems from Variation in the Natural Abundance of ^{15}N

A few results from a collaborative study in the Sonoran desert of Southern California are described below. We worked there with Wes Jarrell from the University of California, Riverside, Ross Virginia from San Diego State University, Phil Rundel from the University of California, Los Angeles, and their colleagues. (Detailed reports of these studies appear elsewhere; e.g., Nilsen et al., 1982; Rundel et al., 1982; Sharifi et al., 1982; Shearer, et al., 1983; Virginia & Jarrell, 1983, Rundel, this volume.)

The ecosystem at the primary study site was dominated by *Prosopis glandulosa* (honey mesquite), which had a very high level of productivity (about 4,000 kg dry wt $ha^{-1}yr^{-1}$—or in terms of N, about 60 kg N $ha^{-1}yr^{-1}$). This 60 kg N $ha^{-1}yr^{-1}$ productivity is for only about 30% cover. Comparing this N productivity to the productivity (about 120 kg N/ha) that a corn-belt farmer takes to the barn as corn makes it even clearer that this is a remarkably productive system—desert or not. Eskew & Ting (1978) and Felker & Clark (1980) showed that mesquite forms effective nodules, so we knew these plants were genetically capable of fixing N_2. Our objective was to determine how much N_2 they were fixing in the particular ecosystems of interest to us and our colleagues. Table 2 summarizes the results of a large number of measurements made on *Prosopis* and non-N_2-fixing reference plants at six sites in the Sonoran desert.

We have accumulated a rich data base on the ^{15}N abundance of N_2-fixing and reference plants in warm desert ecosystems, particularly for our primary study site, Harper's Well. Table 2 shows only 40 measurements made on five *Prosopis* plants and 12 measurements on non-N_2-fixing reference plants at that site. The mean $\delta^{15}N$ value for *Prosopis* at Harper's Well was $+1.2 \pm 0.2‰$. The mean value for the nonfixing reference plants was $+5.0 \pm 0.5‰$. This latter number is the value for the "other N sources" pole. These values are significantly different from each other. The same is true at the other sites shown in Table 2.

Some sites are considerably more favorable for applying the ^{15}N natural abundance method than others. Compare, for example, the values for the reference plants at Clark Dry Lake ($+13.0 \pm 0.4‰$) and the Transect near Harper's Well ($3.0 \pm 0.6‰$). Clearly, the two poles of the interpolation are farther apart at Clark Dry Lake than they are along the Transect near Harper's Well. As

Table 2. $\delta^{15}N$ abundance of *Prosopis* and non-N_2-fixing reference plants at six sites in the Sonoran desert of Southern California.

Site	Plant	n	$\delta^{15}N$—‰
Harper's Well	*Prosopis*	40	$+1.2 \pm 0.2$
	Reference	12	$+5.1 \pm 0.5$
Transect near Harper's Well	*Prosopis*	9	$+1.1 \pm 0.5$
	Reference	15	$+3.0 \pm 0.6$
Nude Wash	*Prosopis*	1	$+1.2$
	Reference	19	$+5.7 \pm 0.3$
Borrego Sink	*Prosopis*	3	$+2.0 \pm 0.2$
	Reference	5	$+6.6 \pm 0.7$
Carrizo Badlands	*Prosopis*	2	$+2.2 \pm 0.5$
	Reference	4	$+6.6 \pm 0.6$
Clark Dry Lake	*Prosopis*	3	$+4.7 \pm 0.7$
	Reference	4	$+13.0 \pm 0.4$

TABLE 3. Estimation of the approximate fractional contribution of biologically fixed N to *Prosopis* at six California Sonoran desert sites. From Shearer et al. (1983).

Site	%N fixed
Harper's Well	61 ± 7
Transect near Harper's Well	43 ± 15
Nude Wash	65
Borego Sink	60 ± 5
Carrizo Badlands	57 ± 6
Clark Dry Lake	58 ± 5

a result, the dispersion of the data around the mean value is a greater fraction of the distance (in $\delta^{15}N$ units) between the "atmospheric N_2" pole and the "soil N" pole in the latter case than it is in the former.

In addition to the data of Table 2, one other number is needed to estimate the fractional importance of biological N_2-fixation; namely, the $\delta^{15}N$ value of the "biologically fixed N" pole. A value of -1.3 ± 0.5‰ was assigned as the value of the "biologically fixed N" pole. This assignment was based on measurements of greenhouse-grown, nodulated *Prosopis* forced to depend on N_2-fixation as its only N source.

The calculated values of %Ndfa are given in Table 3. Overall, at the six California sites, N_2-fixation contributed 40–60% of the N found in *Prosopis*. The error term reflects the error in all three values which go into making the calculation; namely, the ^{15}N abundance of the N_2-fixing plant, of the non-N_2-fixing reference plants and of the N_2-fixing plant grown hydroponically in the absence of any combined N. Note that the real variability in the importance of N_2-fixation across the site will also contribute to the standard error of the mean. The standard error tends to be about 5–10%. The precision of the estimate will depend on two things: how far apart the poles are; and how many samples are collected and measured. The level of precision of the method makes it inappropriate for detecting very small contributions of N_2-fixation.

The standard error may not be an absolutely accurate reflection of the reliability of the measurement. The largest source of error is the imperfect picture one obtains of the "soil N pole" from the measurements on nonfixing reference plants. Collecting as large a variety of reference plants as possible and examining the variability allows us to capture the greatest part, if not all, of the error introduced by this imperfect characterization.

Table 4 shows data from a site in Baja California, Mexico. The data set includes 13 specimens of nine species of non-N_2-fixing non-legumes. Their growth habits range from phreatophytic shrub to shallowly rooted shrubs and herbaceous perennials. The mean $\delta^{15}N$ value for the non-legumes was 9.3‰. Despite the diversity of rooting habits, the standard error for the non-legumes is only 0.5‰. That is, the ^{15}N abundance of the N pool from which these non-legumes were feeding had a relatively homogeneous ^{15}N abundance. While this is reassuring, it should be noted that the range of values was substantial: from 7.2‰ for one *Hymenoclea* (Compositae) specimen to 12.1‰ for *Lycium* (Solanaceae). This is the kind of variation that requires collection of as many specimens as possible. While the heterogeneity of $\delta^{15}N$ values is an empirical question to be investigated at each site, it is not a disabling factor in most cases. It certainly is not disabling in this case.

Look next at the entries for the woody legume, *Acacia greggii*. While nodulation has been reported for many species of *Acacia*, *A. greggii* does not form nodules. Hence in the context of Table 4, *A. greggii* is a non-N_2-fixing reference plant similar to the 13 specimens of non-legumes. Both the non-legumes and *A. greggii* take all of their N from the soil. It is reassuring, therefore, that the mean $\delta^{15}N$ value for leaf tissue from the three *A. greggii* specimens (10.6 ± 0.8‰) is indistinguishable from the mean of the non-legumes. The papilionoid species present a very different picture. Their ^{15}N abundances are uniformly quite close to that of the "atmospheric N_2" pole.

TABLE 4. $\delta^{15}N$ of leaf tissue of plants at Baja California, Mexico (near Cataviña). Modified from Shearer et al. (1983). PS = phreatophytic shrub; A = annual; S = shrub; HP = herbaceous perennial.

Legumes		Non-Legumes		
Species	$\delta^{15}N$	Species	Growth habit	$\delta^{15}N$
Lupinus sp. #1	−0.1	*Hymenoclea monogyra* #1	PS	+7.2
Lupinus sp. #2	+1.6	*Hymenoclea monogyra* #2	PS	+11.6
Dalea spinosa #1	+1.7	*Euphorbia patens*	A	+7.2
Dalea spinosa #2	+3.2	*Viguera deltoides*	HP	+7.4
Astragalus sp.	+2.0	*Aster* sp.	HP	+7.4
Lotus scoparius	+2.7	*Ephedra californica* #1	S	+8.9
		Ephedra californica #2	S	+9.7
All Papilionoideae (mean ± s.e.)	+1.8 ± 0.6	*Atriplex polycarpa* #1	S	+9.1
		Atriplex polycarpa #2	S	+10.6
		Abronia sp.	HP	+9.5
Prosopis glandulosa #1	+7.8	*Croton californicus*	HP	+9.3
Prosopis glandulosa #2	+8.9	*Lycium andersonii* #1	S	+11.3
Prosopis glandulosa #3	+9.1	*Lycium andersonii* #2	S	+12.1
Prosopis glandulosa #4	+9.2			
Prosopis glandulosa #5	+9.7	All non-legumes (mean ± s.e.)		9.3 ± 0.5
All Prosopis (mean ± s.e.)	+8.9 ± 0.3			
Acacia greggii #1	+9.2			
Acacia greggii #2	+11.7			
Acacia greggii #3	+11.1			
All Acacia (mean ± s.e.)	+10.6 ± 0.8			

Clearly they are fixing a large fraction of their N. The surprise in this table is the five *Prosopis* specimens. It is clear from the six California sites that *Prosopis glandulosa* is genetically competent to fix N; at the Baja California site they are not fixing atmospheric N_2. Their mean ^{15}N abundance ($\delta^{15}N = 8.9 \pm 0.3‰$) is indistinguishable from that for the non-legumes ($9.3 \pm 0.5‰$). While we do not have definitive data that establishes the cause of their physiological behavior, these plants had at least one clear phenotypic difference from *Prosopis* plants growing on a different geologic substrate 10 km away. (The latter had thorns and the former did not and hence, might be considered different varieties.) Based on $\delta^{15}N$ measurements, these latter plants appeared to be fixing a large fraction of their N. Whether this apparent varietal difference was the cause of the observed difference in physiology or whether other factors—for example, the absence of the appropriate bacterial symbiont at one site—were responsible has not been established.

Having briefly described the use of ^{15}N abundance for estimating N_2-fixation, we now turn to a description of some of the implications of the ^{15}N data for nodule metabolism.

V. Isotopic Fractionation and Nodule Metabolism

Early on, we observed that, while soybean plants as a whole had an ^{15}N abundance close to that of the atmosphere, their nodules had a substantially elevated ^{15}N abundance. This basic observation is illustrated by the data in Table 5. If the plant as a whole has an ^{15}N abundance close to that of atmospheric N_2 and the nodules have a substantially higher ^{15}N abundance, then one might expect the aboveground parts of the plants to have a substantially lower ^{15}N abundance. However, the data

TABLE 5. Differences in ^{15}N abundance between soybean plant parts and whole plants, $\Delta\delta^{15}N$ ($\delta^{15}N_{plant\ part}$ minus $\delta^{15}N_{whole\ plant}$). $\delta^{15}N_{whole\ plant}$ is the weighted average of all plant parts. The average $\Delta\delta^{15}N$ values are for various plant ages and growth conditions. Modified from Shearer et al. (1980). Differences between whole plant and plant part are significant at the 99 (**) and 99.9% (***) confidence levels (based on a 2-tailed *t*-test for paired samples).

Plant part	n	$\Delta\delta^{15}N$ (‰)
Leaves	58	-0.8 ± 0.2***
Stems	59	-0.5 ± 0.2**
Pods	32	-0.3 ± 0.2
Seeds	32	-0.5 ± 0.2**
All aboveground portions	59	-0.7 ± 0.1***
Roots	59	$+1.3 \pm 0.2$***
Nodules	59	$+8.3 \pm 0.4$***

of Table 5 do not support that expectation. The reason is that only a small fraction of the N (usually less than 5%) is in the nodule.

We have studied the unique ^{15}N enrichment of root nodules rather extensively because it seems likely that it bears on the metabolic pathway of recently fixed N. The early observations are as follows. 1) Only nodules that are actively fixing N_2 are enriched in ^{15}N; ineffective nodules are not enriched (Kohl et al., 1982, 1983). 2) Not all N_2-fixing nodules are enriched in ^{15}N. All ureide-exporting nodules examined to date have been enriched in ^{15}N, but only a few amide-exporting nodules are enriched (see Shearer et al., 1982; Turner & Bergersen, 1983; Steele et al., 1983). 3) Very young nodules are not enriched in ^{15}N, and ^{15}N enrichment increases with nodule maturity (Kohl et al., 1982; Shearer et al., 1984).

On the basis of these and other observations, we have tested a number of hypotheses. Given space constraints, we will describe only three, with the hope of giving the reader some taste for the flavor of the data and the reasoning. When thinking about these hypotheses, please keep in mind that there are only two ways for any bounded structure to become enriched in ^{15}N. Either something depleted in ^{15}N must be exported from it or something enriched in ^{15}N must be imported into it.

The first hypothesis we tested was that ^{15}N depleted material (that is, material enriched in ^{14}N) is lost from the nodule as a result of denitrification (the reduction of nitrate to N_2O, which is usually further reduced to N_2). The relevant background data include the following: 1) some rhizobial strains are capable of denitrification (Rigaud et al., 1973; Zablotowicz et al., 1978); 2) when denitrification occurs, isotopic fractionation usually causes the volatile product(s), N_2 and N_2O, to be depleted in ^{15}N and residual NO_3^- to be enriched in ^{15}N (Wellman et al., 1968; Bryan et al., 1983). According to the hypothesis, the N of this enriched NO_3^- would then be incorporated into nodule tissue. The result would be ^{15}N enriched nodules.

This hypothesis was shown to be incorrect, because a *Rhizobium japonicum* strain (USDA 33) which is effective for N_2-fixation and which produces ^{15}N enriched nodules does not reduce NO_3^- either in free-living culture (Kohl et al., 1982) or in intact nodules (Bryan et al., 1985). In this case, the observed ^{15}N enrichment of the nodule could not result from denitrification since the nodules did not carry out that process.

For some time we favored the hypothesis that isotopic fractionation associated with ureide biosynthesis leaves behind an ^{15}N enriched pool of metabolites from which nodule protein is made. If this hypothesis were correct, then the protein fraction of the host soluble material should be enriched in ^{15}N, since transport compounds are synthesized within host cells. This is perhaps more easily seen by referring to Figure 7. N_2 is fixed in the bacteroid. Ammonia or ammonium ion is exported to the plant host tissue where it is incorporated first into the amide N of glutamine, then transferred to α-ketoglutarate to produce glutamate. Glutamate is then transaminated with the resultant formation of many amino acids. These amino acids are an important part of the nitrogen pool internal to the nodule (indicated as "I_n" in Fig. 7). Pool I_n serves as the source of N for the

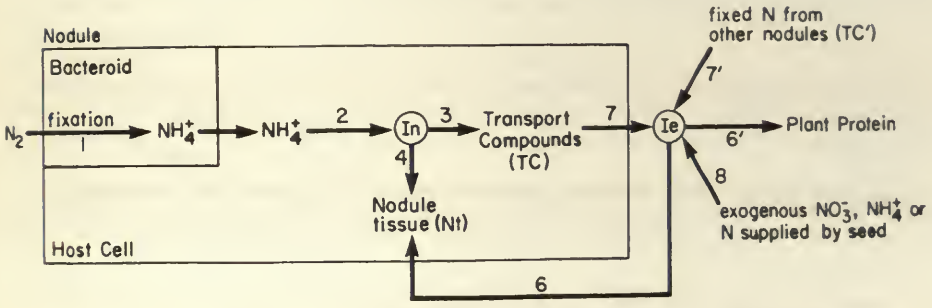

FIGURE 7. A model of the path of fixed N in soybean nodules. N_2 is converted to NH_4^+ within the bacteroid. NH_4^+ (or NH_3) is exported in the host cell where it is assimilated, primarily into glutamate which serves as the basis for many amino acids. These enter into a pool of N-containing intermediates (I_n). Two main fates of molecules in this pool are shown. They can be made into nodule tissue (N_t) or into ureides, the principal N-containing compound transported from the nodule through the xylem to the plant shoot. I_e symbolizes a pool of N-containing intermediates outside of the nodule. Note that this model does not include any provision for the bacteroids to receive the carbon skeletons needed for growth and energy for N_2-fixation.

synthesis of nodule tissue and transport compounds[3]. (In soybeans, most of the fixed N is transported from the nodules to the leaves packaged in compounds called ureides.) If the isotope effect (the quantitative measure of isotopic fractionation) were greater for the synthesis of the transport compounds than for the synthesis of nodule tissue, then pool I_n would become enriched in ^{15}N. Because this pool is used to synthesize nodule proteins, this would result in ^{15}N enriched nodule tissue. Specifically, one would expect the protein of cells originating from the host, as opposed to originating from the symbiont, to be enriched in ^{15}N.

To test this hypothesis, the nodule was separated into three parts. The interior portion was separated from the cortex and then bacteroids were separated from the interior portion. (The cortex is not shown in Fig. 7.) We call the supernatant of the bacteroid pellet the "soluble host material."

Some of the relevant data are shown in Table 6. Contrary to the hypothesis, the soluble host material did not contain the excess ^{15}N which would account for the enrichment of the nodule. The ^{15}N abundance of the nodule was 9.8‰ while the soluble host material was only 4.5‰. In fact, the ^{15}N enrichment was in the bacteroids and the cortex. These results virtually rule out isotopic fractionation associated with the synthesis of transport compounds as the cause of ^{15}N enrichment in soybean nodules.

Since the data show that much of the enrichment in the nodule resides in the bacteroid, we also tested the hypothesis that the bacteroids became enriched in ^{15}N as the result of NH_3 rather than NH_4^+ being exported from the bacteroid. The equilibrium isotope effect for the rapid equilibrium between NH_4^+ and NH_3 is 1.02 (Hermes et al., 1985), leaving NH_4^+ substantially more enriched in ^{15}N than NH_3. Thus, if NH_3 were exported from the bacteroid and the large majority of the N went in that direction, the residual NH_4^+ in the bacteroid would be enriched in ^{15}N. If new bacteroid protein were synthesized from this pool, then the bacteroid protein would become enriched in ^{15}N. The lighter N eventually would be carried off with the ureides, but since the overwhelming majority of the N atoms go in this direction, the ‰ depletion of the transport compounds would be very small. This hypothesis requires the free NH_4^+ pool in the bacteroid to

[3]The actual situation is, of course, more complicated than the schematic picture in Figure 7. One of the complexities omitted is that some of the reactions by which T_c is synthesized take place in the cytosol, while most take place in plastids of infected cells. The final stage of synthesis of the transport compound probably takes place in uninfected nodule host cells (Schubert, 1986).

TABLE 6. Distribution of N and ^{15}N among soybean nodule fractions.

Fraction	% of total nodule N	$\delta^{15}N$ ‰	% of nodule ^{15}N enrichment
Whole nodule	100	+9.8	100
Bacteroids	29	+12.4	55
Cortex	21	+11.1	32
Soluble host material	51	+4.5	13

TABLE 7. ^{15}N abundance of total N and NH_4^+-N of soybean nodules.

Nodule fraction	^{15}Abundance—‰	
	Whole fraction	NH_4^+-N
EXPERIMENT 1		
Bacteroids	+13.2	+4.6
Soluble host material	+4.0	−0.2
Cortex	+8.0	+6.6
EXPERIMENT 2		
Bacteroids	+13.7	+2.3
Soluble host material	+5.2	−3.6
Cortex	+7.7	+1.8

be at least as enriched in ^{15}N as the total bacteroid. The hypothesis also requires a large difference (about 20‰) between the NH_4^+ pools in the bacteroid and the host soluble material.

To test this hypothesis, we measured the ^{15}N abundance of the free NH_4^+ pool in various fractions of soybean nodules (see Table 7). Clearly, the ^{15}N enrichment of the NH_4^+ in the bacteroids cannot account for the enrichment of the bacteroid and hence the $NH_3 + H^+ = NH_4^+$ equilibrium cannot account for the ^{15}N enrichment of the nodule. Also, while the ^{15}N abundance of the NH_4^+-N was significantly different in the bacteroid and host soluble fraction, the difference is not as large as the 20‰ one would expect if the hypothesis were correct. However, the approximately 5‰ difference may indicate that some of the N is exported from the bacteroid as NH_3.

None of the hypotheses we have tested to date account for the observed ^{15}N enrichment of nodules. Thus, at this time the metabolic information contained in the phenomenon remains hidden.

A new, intriguing hypothesis is suggested by Mike Kahn and his colleagues at Washington State University—that organic carbon is supplied to the bacteroid in the form of an amino acid (Kahn et al., 1986). This idea is presented diagramatically in Figure 8. In this figure, the amino acid supplied to the bacteroid is shown as glutamate. (Their idea would be unchanged in its essence if the amino acid entering the bacteroid were a different amino acid, for example, proline.) According to their proposal, once inside the bacteroid, the amino acid is deaminated yielding NH_4^+ and an organic acid. The latter is catabolized to provide the enormous quantity of energy needed by the bacteroid for N_2-fixation. Kahn et al. note that their proposal is supported by theoretical considerations and by the common requirement of many free-living rhizobial strains for glutamic acid, or some other amino acid, in order to achieve maximum rates of N_2-fixation.

The isotopic fractionation associated with the deamination necessary to produce the organic acid from the amino acid is likely to be significant. This deamination of the amino acid would leave behind an ^{15}N enriched pool of the amino acid and an ^{15}N depleted pool of NH_4^+. (The latter would show only a small ‰ ^{15}N depletion since a large majority of the N atoms follow this pathway compared to the competing pathway, namely, to bacteroid protein.) The ^{15}N enriched pool of the

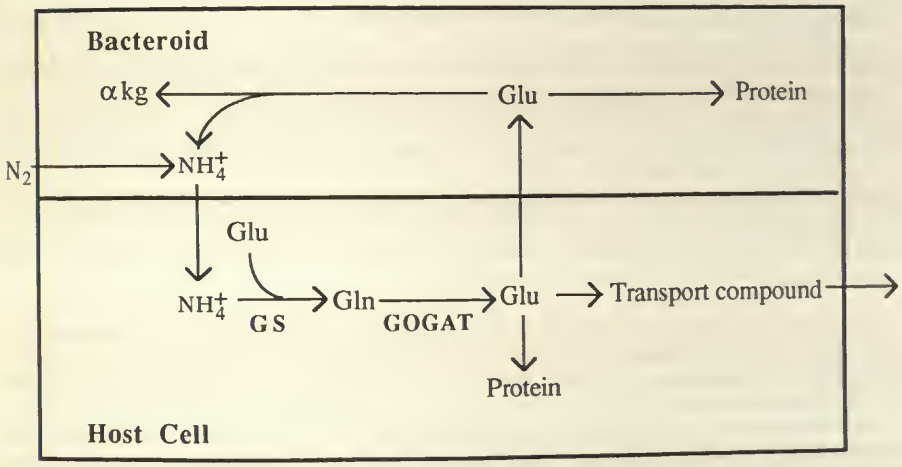

FIGURE 8. A model of the path of fixed N in soybean nodules emphasizing the N and C source of the bacteroid. This model contains the same main features as the model of Figure 7 except that it shows glutamate (made from fixed N) entering the bacteroid where it serves as the N source for bacteroid growth and the C skeleton needed to satisfy the enormous energy needs of N_2-fixation. [Gln = glutamine, GS = glutamine synthetase, Gogat = glutamine-oxoglutarate amino transferase, αkg = α-ketoglutarate (oxoglutarate)]. Modified from Kahn et al. (1985).

amino acid would be used, via transamination reactions, to make the spectrum of amino acids for incorporation into bacteroid protein. The ^{15}N depleted NH_4^+ would be exported to the host cell where it would be assimilated and where transport compounds for export from the nodule are synthesized. This hypothesis is presently being tested.

We are optimistic that we are on the verge of being able to draw conclusions about nodule metabolism from the data generated by studies of isotopic fractionation.

ACKNOWLEDGMENTS

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REFERENCES

BORMANN, F. H., G. E. LIKENS & J. M. MELILLO. 1977. N budget for an aggrading northern hardwood forest ecosystem. Science 196: 981–983.

BRYAN, B. A., G. SHEARER, J. SKEETERS & D. H. KOHL. 1983. Variable expression of the nitrogen isotope effect associated with denitrification of nitrite. J. Biol. Chem. 258: 8613–8617.

———, ———, ——— & ———. 1985. Denitrification by intact soybean nodules in relation to natural ^{15}N enrichment of nodules. Canad. J. Soil Sci. 65: 261–267.

DOMENACH, A. M. & A. CORMAN. 1984. Dinitrogen fixation by field grown soybeans; statistical analysis of variation in $\delta^{15}N$ and proposed sampling procedures. Pl. & Soil 78: 301–313.

ESKEW, D. L. & I. P. TING. 1978. Nitrogen fixation by legumes and blue-green algal-lichen crusts in a Colorado desert environment. Amer. J. Bot. 65: 850–856.

FELKER, P. & P. R. CLARK. 1980. Nitrogen fixation (acetylene reduction) and cross inoculation in 12 *Prosopis* (mesquite) species. Pl. & Soil 57: 177–186.

——— & ———. 1982. Position of mesquite (*Prosopis* spp.) nodulation and nitrogen fixation (acetylene reduction) in 3-m long phreatophytically simulated soil columns. Pl. & Soil 64: 297–305.

———, G. H. CANNELL & P. R. CLARK. 1981. Variation in growth among 13 *Prosopis* (mesquite) species. Expl. Agric. 17: 209–218.

Hansen, A. P., J. S. Pate & C. A. Atkins. 1987. Relationship between acetylene reduction activity, hydrogen evolution and nitrogen fixation in nodules of Acacia spp. J. Exptl. Bot. 38: 1–12.

Hermes, J., P. Weiss & W. W. Cleland. 1985. The use of ^{15}N and deuterium isotope effects to determine the chemical mechanism of phenylalanine ammonia lyase. Biochemistry 24: 2959–2967.

Kahn, M. L., J. Kraus & J. E. Somerville. 1985. A model of nutrient exchange in the rhizobium-legume symbiosis. Pp. 193–199 *in* H. J. Evans, P. J. Bottomley & W. E. Newton (editors), Nitrogen Fixation Research Progress. Martinus Nijhoff, Dordrecht.

Kohl, D. H. & G. Shearer. 1981. The use of soils lightly enriched in ^{15}N to screen for N_2-fixing activity. Pl. & Soil 60: 487–489.

———, B. A. Bryan & G. Shearer. 1983. Relationship between N_2-fixing efficiency and natural ^{15}N enrichment of soybean nodules. Pl. Physiol. 73: 514–516.

———, ———, L. Feldman, P. H. Brown & G. Shearer. 1982. Isotopic fractionation in soybean nodules. Pp. 9–33 *in* H. L. Schmidt, H. Förstel & K. Heinzinger (editors), Stable Isotopes: 4th International Conference. Elsevier, Amsterdam.

Mariotti, A., F. Mariotti, N. Amarger, G. Pizelle, J. M. Ngambe, M. L. Champigny & A. Moyse. 1980. Fractionments isotopique de l'azote lors des processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plants. Physiol. Veg. 18: 163–181.

Nilsen, E. T., P. W. Rundel & M. R. Sharifi. 1981. Summer water relations of the desert phreatophyte *Prosopis glandulosa* in the Sonoran Desert of southern California. Oecologia Berl. 50: 271–276.

Rigaud, J., F. J. Bergersen, G. L. Turner & R. M. Daniel. 1973. Nitrate dependent anaerobic aceytlene-reduction and nitrogen-fixation in soybean bacteroids. J. Gen. Microbiol. 77: 137–144.

Rundel, P. W., E. T. Nilsen, M. R. Sharifi, R. A. Virginia, W. M. Jarrell, D. H. Kohl & G. B. Shearer. 1982. Seasonal dynamics of nitrogen cycling for a *Prosopis* woodland in the Sonoran Desert. Pl. & Soil 67: 343–353.

Schubert, K. R. 1986. Products of biological nitrogen fixation in higher plants: Synthesis, transport, and metabolism. Ann. Rev. Pl. Physiol. 37: 539–574.

Sharifi, M. R., E. T. Nilsen & P. W. Rundel. 1982. Biomass and net primary production of *Prosopis glandulosa* (Fabaceae) in the Sonoran Desert of California. Amer. J. Bot. 69: 760–768.

Shearer, G. & D. H. Kohl. 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Austral. J. Plant Physiol. 13: 699–756.

——— & ———. 1988. Estimates of N_2-fixation in ecosystems: the need for and basis of the ^{15}N abundance method. Pp. 342–374 *in* P.W. Rundel, J.R. Ehleringer & K.A. Nagy (editors), Stable Isotopes in Ecological Research. Springer-Verlag, New York.

———, B. A. Bryan & D. H. Kohl. 1984. Increase of natural ^{15}N enrichment of soybean nodules with mean nodule mass. Pl. Physiol. 76: 734–746.

———, D. H. Kohl & S. H. Chien. 1978. The nitrogen-15 abundance in a wide variety of soils. Soil Sci. Soc. Amer. J. 42: 899–902.

———, ——— & J. E. Harper. 1980. The distribution of ^{15}N among plant parts of nodulating and non-nodulating isolines of soybeans. Pl. Physiol. 66: 57–60.

———, ———, R. A. Virginia, B. A. Bryan, J. L. Skeeters, E. T. Nilsen, M. R. Sharifi & P. W. Rundel. 1983. Estimates of N_2-fixation from variation in the natural abundance of ^{15}N in Sonoran Desert ecosystems. Oecologia Berl. 56: 365–373.

Steele, K. W., B. M. Bonish, R. M. Daniel & G. W. O'Hara. 1983. Effect of rhizobial strains and host plant on nitrogen isotopic fractionation in legumes. Pl. Physiol. 72: 1001–1004.

Turner, G. L. & F. J. Bergersen. 1983. Natural abundance of ^{15}N in root nodules of soybean, lupin. subterranean clover and lucerne. Soil Biol. Biochem. 15: 525–530.

——— & A. H. Gibson. 1980. Measurement of N_2 fixation by indirect means. Pp. 111–138 *in* F. J. Bergersen (editor), Methods for Evaluating Biological Nitrogen Fixation. John Wiley & Sons, New York.

Virginia, R. A. & W. M. Jarrell. 1983. Soil properties in a mesquite-dominated Sonoran Desert ecosystem. Soil. Sci. Soc. Amer. J. 47: 138–144.

———, ———, P. W. Rundel, G. Shearer & D. H. Kohl. 1988. The use of variation in the natural abundance of ^{15}N to assess symbiotic N_2-fixation by woody plants. Pp. 375–394 *in* P.W. Rundel, J.R. Ehleringer & K.A. Nagy (editors), Stable Isotopes in Ecological Research. Springer-Verlag, New York.

Wellman, R. P., F. D. Cook & H. R. Krouse. 1968. Nitrogen-15: Microbial alteration of abundance. Science 161: 269–270.

Witty, J. F. 1983. Measurement of N_2-fixation by ^{15}N fertiliser dilution; Problems of declining soil enrichment. Pp. 253–266 *in* D.G. Jones & D.R. Davies (editors), Temperate legumes: physiology, genetics and nodulation. Pitman Advanced Publishing Program, Melbourne.

Zablotowicz, R. M., D. L. Eskew & D. D. Focht. 1978. Denitrification in rhizobium. Canad. J. Microbiol. 24: 757–760.

INTERACTIONS BETWEEN HERBACEOUS LEGUMES AND THEIR ASSOCIATED RHIZOBIA, MYCORRHIZAE, PESTS, AND PARASITES

N. C. Schenck[1]

Abstract

Soil has a complex ecosystem in which the interrelationships of organisms are not well understood. However, in the last 30 years there has been considerable interest in evaluating interactions of organisms occurring in soil. Mycorrhizal fungi are one group of soil organisms receiving recent study. Several different types of these fungi form symbiotic relationships with legumes, but the most common are the vesicular-arbuscular (VA) mycorrhizal fungi. The VA mycorrhizal fungi have generated much interest because of the beneficial attributes they can confer to plants, such as their ability to increase plant absorption of phosphorus, an element frequently found in soils in amounts that limit plant growth. The VA mycorrhizal fungi have increased nodulation and nitrification in some legumes in laboratory and field tests, but the range of responses varies. Most legumes receive considerable benefit, but in some instances actual plant growth depressions occur. Interactions occur among rhizobia, VA mycorrhizal fungi, and plant pathogens associated with legumes. Although the interactions are complex and difficult to study, rhizobia and mycorrhizal fungi have been shown to reduce the effects of root pathogens on legumes. The use of mycorrhizal fungi to improve plant growth, nodulation, and nitrification in legumes is currently limited. A readily available inoculum for large-scale application of VA mycorrhizal fungi in the field cannot be provided at this time. The need for additional studies in the interrelationships of rhizobia and VA mycorrhizal fungi on legumes is suggested because of their combined potential value and use in commercial legume production.

I. Introduction

Several different types of mycorrhizal fungi form symbiotic associations with plant roots (Harley & Smith, 1983). By far the most common are the vesicular-arbuscular (VA) mycorrhizal fungi, so called because of the structures formed in roots termed vesicles and arbuscules (Fig. 1; Nicolson, 1967). Approximately 120 described species of these fungi exist and they are all in the order Endogonales (Zygomycetes). The VA mycorrhizal fungi are among the most common soil fungi and are obligately associated with roots of plants. They have little, if any, independent existence in soil. The VA micorrhizal fungi have broad host ranges; the same fungus can establish in the roots of plants as diverse as ferns, giant sequoias, and legumes.

Considerable interest has been generated in VA mycorrhizal fungi because of the attributes they can confer to plants. Most research has been centered on their ability to increase plant absorption of phosphorus from soils in which the availability of phosphorus is limited (Mosse, 1973). The fungus can extend its filamentous hyphae from the root into the soil beyond the limits of root hairs. Phosphorus can be translocated in the hyphae to the plant and then transferred to the plant cell from the arbuscle, a finely branched, haustorium-like structure. There is evidence that the fungus can translocate copper (Gildon & Tinker, 1983b) to plants in a similar manner and speculation that a similar function may exist for translocating other relatively immobile elements in the soil. Mycorrhizal plants in a soil limited in available phosphorus or copper have an advantage over plants lacking mycorrhiza (Plenchette, 1983; Yost & Fox, 1979). Mycorrhizal plants also have been shown experimentally to be more tolerant to drought (Nelson & Safir, 1982), root pathogenic fungi and

[1]Plant Pathology Department, University of Florida, Gainesville, Florida 32611, U.S.A.

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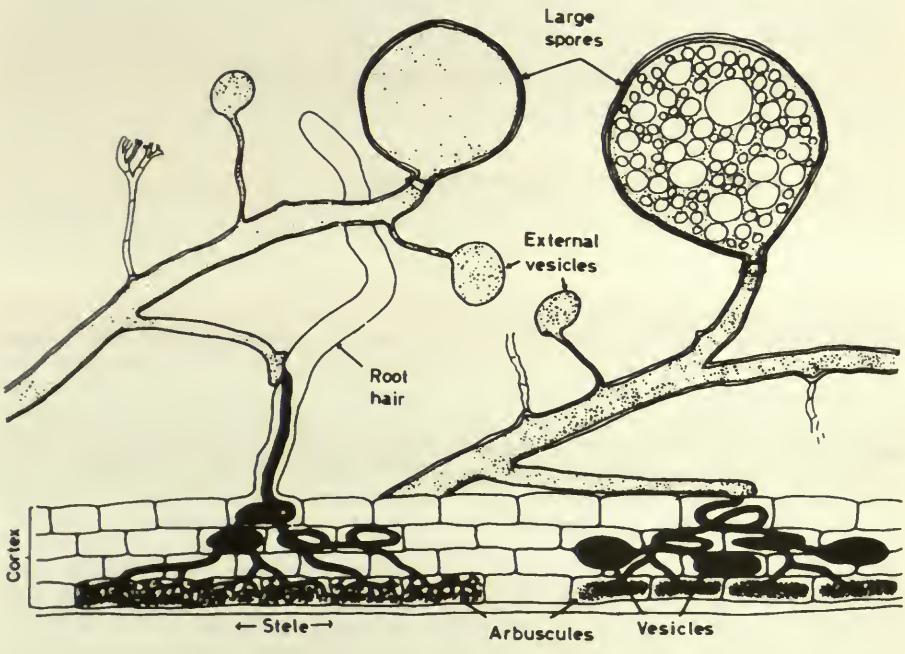

FIGURE 1. Diagram of the structures formed inside and outside a root by a typical vesicular-arbuscular mycorrhizal fungus (from Nicolson, 1967).

nematodes (Schenck, 1981), saline soils (Pond et al., 1984), high soil temperature (Schenck & Schroder, 1974), toxic heavy metals in soil (Gildon & Tinker, 1983a), and several other stressful conditions. In experimental conditions, plant growth increases of as high as 13,000% have been reported with mycorrhizal fungi. Some plants are so dependent on mycorrhizal fungi, they can not grow without them (Plenchette, 1983).

II. INTERACTIONS

RHIZOBIA AND VA MYCORRHIZAE

It has been known for some time that VA mycorrhizal fungi can increase growth and nodulation of most legumes. Asai (1944) demonstrated that 54 species of legumes were stimulated in growth by the addition of small quantities of garden humus soil to steam sterilized soils. Asai showed this before the effect of VA mycorrhiza on legumes was known widely, and the effect was ascribed to "detoxification" of steamed soil (Mosse, 1976). This response is best explained by the addition of mycorrhizal fungi that were carried with the garden humus (Gerdemann, 1968). Since then additional researchers have shown repeatedly that legumes benefit from colonization by VA mycorrhizal fungi (Mosse, 1976), especially in soils low in available phosphorus.

Not all legume species benefit equally from the presence of VA mycorrhizal fungi in their roots, however. Asai (1944) noted that the response of lupines and *Vicia sativa* was much less than for other legumes. *Lupinus consentinii* in Australia was found to have only little root colonization with VA mycorrhiza (Trinick, 1977) despite the fact it established readily in low phosphorus soils.

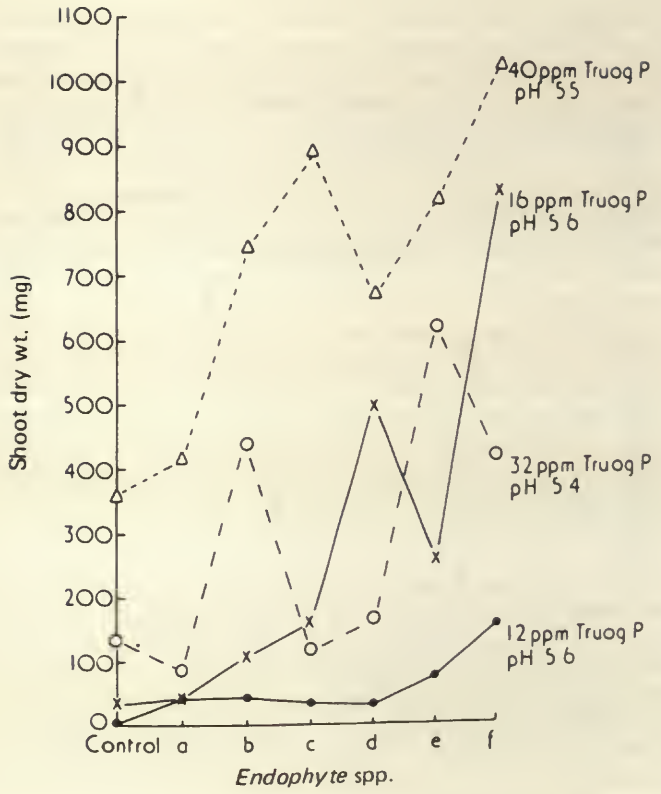

FIGURE 2. Response of white clover *(Trifolium repens)* to six species of mycorrhizal fungi (*Endophyte* spp.) in four different soil types differing in available phosphorus (from Mosse, 1981). Control = no mycorrhizae, a = *Glomus mosseae*, b = *Glomus* sp., c = *Acaulospora laevis*, d = *Gigaspora margarita*, e = indigenous fungi, f = *Glomus fasciculatus* (E_3).

Differences in legume species and their responses to VA mycorrhiza depend on their capacity to absorb phosphorus in the nonmycorrhizal condition which could be affected by root hair length, root growth habit, and their innate phosphorus requirement (Munns & Mosse, 1980).

As stated above, VA mycorrhizal fungi have extensive host ranges, and a single legume species could be colonized by a number of different VA mycorrhizal fungi. However, not all species of mycorrhizal fungi have the same effect on the host. Some species of mycorrhizal fungi may promote nodulation and plant growth on some legume species and have little or no effect on others. Such reactions have been reported by Skipper & Smith (1979) for soybean, Ollivier et al. (1983) for *Vigna unguiculata*, and Powell (1977) for white clover. Mosse (1981) summarized data for white clover (Fig. 2). The nature of this specificity is not known but has been shown to occur on a variety of hosts and could be influenced by many host, fungus, soil, and environmental factors.

Another factor affecting legume response to VA mycorrhizal fungi is the amount of available phosphorus in the soil (Mosse, 1976). When phosphorus levels in the soil are high, there is frequently no benefit from VA mycorrhiza, which in fact may be detrimental (Asimi et al., 1980; Crush & Caradus, 1980). Several authors indicate that high levels of phosphorus in the host reduce the incidence and hence the effect of mycorrhizal fungi on their host (Daft & Nicolson, 1969; Johnson, 1976; Khan, 1972).

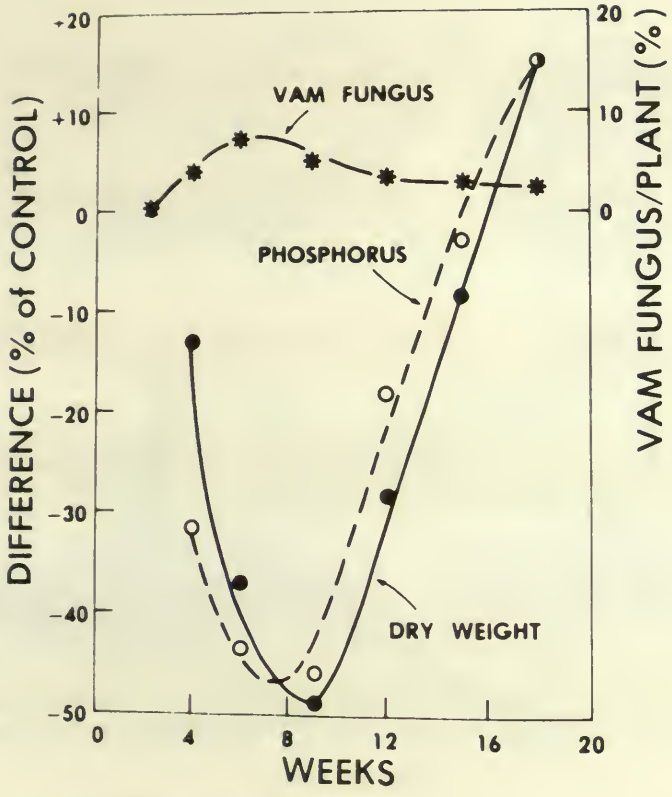

FIGURE 3. Effect of the mycorrhizal fungus *Glomus fasciculatum* on the dry weight and phosphorus content of soybean *(Glycine max)* expressed as percent of nonmycorrhizal control soybeans over time (from Bethlenfalvay et al., 1982).

Both symbionts obtain their carbon source from the host legume. This could result adversely on the host under certain conditions. The exact amount of carbon required by both symbionts is not known specifically but estimates have been placed at near 10% of the total carbon produced by the host (Paul & Kucey, 1981; Pang & Paul, 1980; Snellgrove et al., 1982). Under conditions of stress for light (Furlan & Fortin, 1973) and/or temperature (Schenck & Schroder, 1974), mycorrhizal plants can have reduced growth compared to nonmycorrhizal plants. At four to 12 weeks of soybean growth, Bethlenfalvay et al. (1982) obtained a temporary growth depression in mycorrhizal plants compared to nonmycorrhizal plants (Fig. 3). This depression happened when maximum development of the fungal symbiont was occurring. Bethlenfalvay et al. suggested that carbohydrates demanded by the fungus when the photosynthetic source capacity of the host was low resulted in this growth depression. Bayne et al. (1984) further demonstrated that the fungus will develop even at the expense of *Rhizobium*. Using defoliation to create a carbon stress on soybean and its symbionts, Bayne et al. observed relatively no change in the biomass of mycorrhizal fungi but considerable reduction in nodule weight and activity (as measured by H_2 evolution) with the defoliation treatment.

Although the interactions of VA mycorrhizal fungi and legumes can be affected by several factors, by far the most common response is positive. This positive response results primarily from the increase in phosphorus absorption by the VA mycorrhizal fungi. This is due probably to the

additional requirement for phosphorus in the nodule, which has a two to three times greater P–level requirement than the root (Mosse, 1981). However, additional effects have been hypothesized since in many instances phosphorus applications alone do not produce an effect equal to that of mycorrhizal inoculation (Daft & El-Giahmi, 1974). Other elements also are required for nodule development; i.e., zinc and copper, and mycorrhizal fungi have been shown to increase absorption of these compounds as well (Munns & Mosse, 1980; Gilmore, 1971).

The effect of the mycorrhizae on nodulation is rapid, often occurring within the first few weeks after inoculation (Smith & Daft, 1977). Both Asimi et al. (1980) and Oliver et al. (1983) noted that the mycorrhizal effect on nodulation and nitrogenase activity was rapid and occurred even in plants receiving additional phosphorus applications adequate to eliminate growth differences between mycorrhizal and nonmycorrhizal plants. This would indicate that the phosphorus effects on plant growth are less demanding than the phosphorus requirements for good *Rhizobium* activity and that the first phosphorus effects of the mycorrhizal fungus occur in the nodules. A level of 0.25 g/kg soil of phosphorus was required to eliminate plant growth differences, while a phosphorus level of 0.5 g/kg soil and 1.0 g/kg soil were necessary to eliminate mycorrhizal effects on nodule formation and nitrogenase activity, respectively (Asimi et al., 1980). The inflow of phosphorus in mycorrhizal and nonmycorrhizal plants was shown to be the same except at the very earliest (up to 30 days) sampling dates (Smith, 1982). This increased early phosphorus inflow may explain the rapid effect on nodulation found in previous studies. Obviously, the effect of mycorrhiza on nodulation and nitrogen fixation is not completely understood at this time.

The stimulatory effect on legumes occurred not only in the greenhouse but also in the field. Powell (1976) noted that in 27 of 37 New Zealand hill country soils inoculation with mycorrhizae increased white clover *(Trifolium repens)* growth. With eight soils, mycorrhizal inoculation resulted in over 100% growth increases; in 20 soils Powell obtained growth increases over 30%. Bagyaraj et al. (1979) obtained greater nodulation and nitrogen fixation with soybean after dual inoculation with a mycorrhizal fungus and *Rhizobium japonicum* than either symbiont alone in an Indian soil with low available phosphorus (2.24 ppm). Cheng et al. (1984) also obtained increased (3–8%) soybean seed yield with inoculation of VA mycorrhizal fungi in Taiwan field soils. Hayman et al. (1981) in England obtained increased plant growth of red clover *(Trifolium pratense)* using several methods of mycorrhizal inoculation. Rangeley et al. (1982) in hill country soils in Scotland obtained a growth increase of 100% with white clover after inoculation with VA mycorrhizal fungi but not until the second year of growth (first harvest year). Kucey & Diab (1984) obtained increases in growth of alfalfa *(Medicago sativa)* in Canada after inoculation but only in limed soils that favored the nonindigenous mycorrhizal inoculum source over the indigenous mycorrhizal fungi. Elmes et al. (1984) used mycorrhizal bean *(Phaseolus vulgaris)* roots as inoculum for red clover in the field and obtained significant shoot growth increases. Many soil types were included in these tests but all soils evaluated had low (2.4–14 ppm) amounts of available phosphorus. These legume responses were obtained with several different species of mycorrhizal fungi. In some cases the growth and/or yield increases were substantial, indicating the importance of adequate mycorrhizal colonization of legume roots in the field for good plant growth.

PLANT PARASITES, PATHOGENS, AND VA MYCORRHIZAE

Over 75 publications have dealt with the effect of VA mycorrhizal fungi on plant diseases. Most of these have dealt with the effect on diseases caused by soilborne pathogens or parasites. There are reviews of this subject by Schenck (1981), Dehne (1982), and Hussey & Roncadori (1982). Although in the majority of instances, mycorrhizal fungi used in these tests had a positive effect (reduced disease severity or pathogen incidence) on disease, there are several instances where no effect or a negative effect (increased disease severity or pathogen incidence) occurred. A portion of

this variability may have resulted from the use of different systems of hosts, pathogens, and mycorrhiza in the various studies. However, the procedures used in each study may have had an equal effect since methods of inoculation, quantities of inoculum, and age of the host when challenged with the pathogen can affect results (Schenck, 1987).

The reason for the effect of mycorrhiza on disease is not well known but several suggestions have been postulated. Increases in phosphorus levels in mycorrhizal plants have been suggested as being responsible for increasing (Davis et al., 1979) and decreasing (Graham & Menge, 1982) plant response to pathogens. The pathogen *Verticillium dahliae*, a wilt-producing fungus, was stimulated by phosphorus; the pathogen *Gaeumannomyces graminis* var. *tritici*, a root-invading fungus, was suppressed by phosphorus. Other factors suggested as being responsible for the "mycorrhizal effect" on plant disease are: an increase in root amino acid levels (Baltruschat & Schönbeck, 1975); increase in root sucrose levels (Safir, 1968); increase in phenolic compounds (Krishna & Bagyaraj, 1983); increase in wound barrier formation in roots (Wick & Moore, 1984); and greater lignification on the cell wall (Dehne & Schönbeck, 1979b).

The reduction in disease by mycorrhizae has been observed only with soilborne pathogens and parasites. The incidence and severity of disease caused by foliar-infecting fungi and viruses appear to be increased by mycorrhizae (Schönbeck & Dehne, 1979), but the number of studies with foliar-infecting organisms has been few.

Dehne & Schönbeck (1975, 1979a), who studied the effect of a mycorrhizal fungus on Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, observed a significant reduction in the wilt index (2.9–1.1) on mycorrhizal versus nonmycorrhizal plants. Davis & Menge (1981) noted a suppression in the effect of *Phytophthora parasitica*, a root-rotting fungus, on sweet orange citrus seedlings when plants were mycorrhizal, but the pathogen at high inoculum levels negated the beneficial effects. Zambolim & Schenck (1983) were able to completely negate the effect of three root pathogens *(Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium solani)* by inoculating soybean plants with a mycorrhizal fungus *(Glomus mosseae)* in both steamed and field soil (Fig. 4). Working with root-knot nematode *(Meloidogyne incognita)* on cotton *(Gossypium hirsutum;* Malvaceae), Smith et al. (1986) reduced the severity of yield losses and reduced the juvenile nematode population when inoculating plants with mycorrhizal fungi.

RHIZOBIA, PATHOGENS, AND MYCORRHIZAE

Few papers report the complex interactions of rhizobia-mycorrhizae-pathogen combinations. The complexities of the study and the problem of eliminating the desired organisms in the appropriate controls present obstacles that discourage most casual investigators of these relationships. In one of the first studies evaluating VA mycorrhizal fungi on a legume (soybean) with a plant pathogen *(Phytophthora megasperma* var. *sojae)*, Ross (1972) noted that mycorrhizal soybeans at maturity had more root rot than nonmycorrhizal soybeans. Chou & Schmitthenner (1974), in a complex study involving *Rhizobium*, mycorrhizae, and two pathogens on seedling soybeans, observed that mycorrhizal *(Glomus mosseae)* inoculated plants had greater dry weights and fewer dead plants from *P. megasperma* var. *sojae* than nonmycorrhizal plants. The presence of *Rhizobium* had little effect on the interaction between soybean and the pathogens. The results from these two studies established that mycorrhizal fungi affect host reaction to other rhizosphere microorganisms, while rhizobia in the Chou & Schmitthenner study had little effect on them.

Schenck & Hinson (1973) were the first to use nodulating and nonnodulating isolines of soybean ('Hardee') to evaluate the effect of *Rhizobium* on interactions of legumes with other microorganisms. After mycorrhizal inoculation, Schenck & Hinson noted significant effects on plant growth and yield of nodulated soybeans but not nonnodulated soybeans. The yields of nonnodulated soybeans were poor in unfumigated soil, indicating a possible adverse effect of a pathogen that was

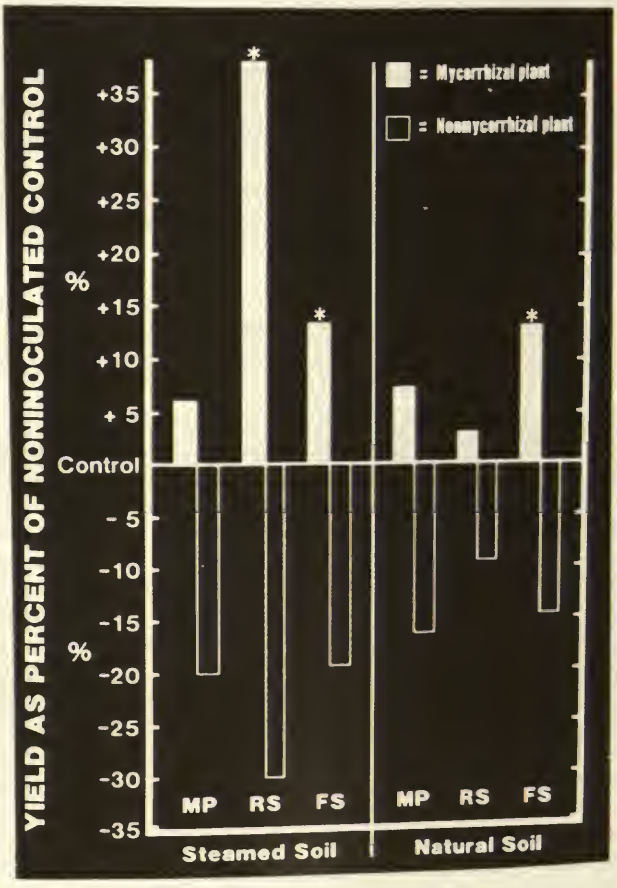

FIGURE 4. Effect of inoculation with *Glomus mosseae* on soybean yield depression by three root-infecting fungi, MP = *Macrophomina phaseolina*, RS = *Rhizoctonia solani*, and FS = *Fusarium solani* in steamed and natural soils (from Schenck, 1981).

not as detrimental to nodulated soybeans. Some years later, Tu (1978a, b) demonstrated that the presence of *Rhizobium* in soybean can have a marked effect on *Fusarium oxysporum* and *Phytophthora megasperma* var. *sojae*. Perhaps this type of effect occurred in the study by Schenck & Hinson (1973).

One of the most recent and detailed studies involving complex interactions of several microorganisms with *Rhizobium* is that of Zambolim & Schenck (1984). In this study, nodulating and nonnodulating soybeans were compared with and without a mycorrhizal fungus and three root pathogens (*Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani*). Although the results indicated that the pathogens were not affected differently by the mycorrhizal fungus (*Glomus mosseae*) on the two isolines, there were some differences between isolines with two of the pathogens (*M. phaseolina* and *R. solani*). The effect of the pathogens was significantly less as measured by reduced shoot weight on the nodulating than the nonnodulating isoline. Since all seeds were inoculated with *Rhizobium*, this suggested that the presence of *Rhizobium* in the nodule affects the plant response to a pathogen in conjunction with the mycorrhizal fungus.

III. Conclusions and Suggestions

Evidence indicates that VA mycorrhizal fungi can increase growth, nodulation, and nitrification in legumes. This area of study may be fruitful in areas of the world where available phosphorus is limited. In one such area, the Llanos of eastern Colombia, attempts are being made to increase pasture legume productivity through use of mycorrhizae (Salinas et al., 1985) in order to improve the cattle and beef production in this area. Soil in the Llanos is an acid oxisol, which forms iron and aluminum complexes with added phosphorus fertilizers, thus preventing any long-term advantage from phosphorus fertilization. In such areas, the better utilization of existing soil phosphorus by mycorrhizal fungi has considerable potential.

The use of mycorrhizal fungi in commercial agriculture is restricted because of a lack of suitable amounts of inoculum. These fungi are obligate symbionts, which will not grow extensively or reproduce abundantly except on the roots of living plants. Inocula produced by current methods have been utilized successfully in field studies and are feasible in several instances. The evaluation of methods for producing mycorrhizal inoculum will continue and I predict that eventually a method for rapid production of mycorrhizal fungi will be found. Until this time, their application will be limited to situations requiring only small amounts of inoculum (Menge, 1983).

The use of mycorrhizae in commercial agriculture on legumes and other crops was the subject of a recent symposium in Florida (Ferguson, 1984). The potential for use in agriculture of VA mycorrhizae is limited in developed countries with intensive agriculture systems employing extensive use of fertilizers, especially phosphorus. Most mycorrhizal fungi do not develop extensively in their hosts in high fertility soils. Because the main benefit to plants seems to be increased absorption of phosphorus, all other attributes conveyed to plants (see the beginning of this chapter) by mycorrhizal fungi are considered secondary (Rhodes, 1984). Factors limiting their use in the United States according to Menge (1984) are: 1) the lack of high-quality inoculum and a market for large-scale production of VA mycorrhizal fungi; 2) the lack of publicized large-scale field tests; and 3) the lack of evidence for cost-effectiveness of using mycorrhizae. The need for greater efforts to evaluate the use of mycorrhizae in the field and overcome some of the above shortcomings is apparent. The need for use of mycorrhizae in agriculture is perhaps more acute in developing countries. Several countries are evaluating the use of mycorrhizae with legumes in the field, including the Philippines, Thailand, Malaysia, India, and Brazil. The apparent need of legumes for mycorrhizae in soils with low phosphorus availability should stimulate our interests and efforts in utilizing mycorrhizae on legumes in commercial agriculture.

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References

Asai, T. 1944. Über die Mykorrhizen bildung der Leguminosen-Pflanzen. Jap. J. Bot. 13: 463–485.

Asimi, A., V. Gianinazzi-Pearson & S. Gianinazzi. 1980. Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and *Rhizobium* in soybeans. Canad. J. Bot. 58: 2200–2205.

Bagyaraj, D. J., A. Manjunath & R. B. Patil. 1979. Interactions between a vesicular-arbuscular mycorrhiza and *Rhizobium* and their effects on soybean in the field. New Phytol. 82: 141–145.

Baltruschat, H. & F. Schönbeck. 1975. The influence of endotrophic mycorrhiza on the infestation of tobacco by *Thielaviopsis basicola*. Phytopathol. Z. 84: 172–188.

Bayne, H. G., M. S. Brown & C. J. Bethlenfalvay. 1984. Defoliation effects on mycorrhizal colonization, nitrogen fixation and photosynthesis in the *Glycine-Glomus-Rhizobium* symbiosis. Physiol. Plantarum 62: 576–580.

Bethlenfalvay, G. J., M. S. Brown & R. S. Pacovsky. 1982. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: development of the host plant. Phytopathology 72: 889–892.

Cheng, Y. H., C. C. Tu & J. C. Lin. 1984. Effect of VA mycorrhizae on yield production of soybean and corn. Res. Bull. Tainan 18: 29–38.

Chou, L. G. & A. P. Schmitthenner. 1974. Effects of *Rhizobium japonicum* and *Endogone mosseae* on soybean root rot caused by *Pythium ultimum* and *Phytophthora megasperma* var. *sojae*. Plant Dis. Reptr. 58: 221–225.

Crush, J. R. & J. R. Caradus. 1980. Effect of mycorrhizas on growth of some white clovers. New Zealand J. Agric. Res. 23: 233–237.

Daft, M. J. & A. A. El-Giahmi. 1974. Effect of Endogone mycorrhiza on plant growth. VII. Influence of infection on the growth and nodulation in French beans *(Phaseolus vulgaris)*. New Phytol. 73: 1139–1147.

——— & T. H. Nicolson. 1969. Effect of Endogone mycorrhiza on plant growth II. Influence of soluble phosphate on endophyte and host in maize. New Phytol. 68: 945–952.

Davis, R. M. & J. A. Menge. 1981. *Phytophthora parasitica* inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. New Phytol. 87: 705–715.

———, ——— & D. Erwin. 1979. Influence of *Glomus fasciculatus* and soil phosphorus on Verticillium wilt of cotton. Phytopathology 69: 452–456.

Dehne, H. W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115–1119.

——— & F. Schönbeck. 1975. The influence of the endotrophic mycorrhiza on the fusarial wilt of tomato. Z. Pflanzenkr. Pflanzensch. 82: 630–633.

——— & ———. 1979a. The influence of endotrophic mycorrhiza on plant diseases. I. Colonization of tomato plants by *Fusarium oxysporum* f. sp. *lycopersici*. Phytopath. Z. 95: 105–110.

——— & ———. 1979b. The influence of endotrophic mycorrhiza on plant diseases. II. Phenol metabolism and lignification. Phytopath. Z. 95: 210–216.

Elmes, R. P., C. M. Hepper, D. S. Hayman & J. O'Shea. 1984. The use of vesicular-arbuscular mycorrhizal roots grown by the nutrient film technique as inoculum for field sites. Ann. Appl. Biol. 104: 437–441.

Ferguson, J. J. (editor). 1984. Applications of mycorrhizal fungi in crop production. Instit. Food & Agric. Sciences. Univ. Florida, Gainesville, Florida.

Furlan, V. & J. A. Fortin. 1973. Formation of endomycorrhiza by *Endogone calospora* on *Allium cepa* under three temperature regimes. Naturaliste Canad. 102: 663–667.

Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annual Rev. Phytopath. 6: 397–418.

Gildon, A. & P. B. Tinker. 1983a. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol. 95: 247–261.

——— & ———. 1983b. Interactions of vesicular-arbuscular mycorrhizal infections and heavy metals in plants. II. The effects of infection on uptake of copper. New Phytol. 95: 263–268.

Gilmore, A. E. 1971. The influence of endotrophic mycorrhizae on the growth of peach seedlings. J. Amer. Soc. Hort. Sci. 96: 35–38.

Graham, J. H. & J. A. Menge. 1982. Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat. Phytopathology 72: 95–98.

Harley, J. L. & S. E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press, New York.

Hayman, D. S., E. J. Morris & R. J. Page. 1981. Methods for inoculating field crops with mycorrhizal fungi. Ann. Appl. Biol. 99: 247–253.

Hussey, R. S. & R. W. Roncadori. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. Plant Dis. 66: 9–14.

Johnson, P. N. 1976. Effect of soil phosphate level and shade on plant growth and mycorrhizas. New Zealand J. Bot. 14: 333–340.

KHAN, A. G. 1972. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals I. Effects of maize growth. New Phytol. 71: 613–619.

KRISHNA, K. R. & D. J. BAGYARAJ. 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Canad. J. Bot. 61: 2349–2351.

KUCEY, R. M. N. & C. E. S. DIAB. 1984. Effects of lime, phosphorus and addition of vesicular-arbuscular (VA) mycorrhizal fungi on indigenous VA fungi and on growth of alfalfa in a moderately acidic soil. New Phytol. 98: 481–486.

MENGE, J. A. 1983. Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture. Canad. J. Bot. 61: 1015–1024.

_____. 1984. The feasibility of using VA mycorrhizal fungi to improve crop production in fumigated and nonfumigated soil. Pp. 29–34 *in* J. J. Ferguson (editor), Application of Mycorrhizal Fungi in Crop Production. Instit. Food & Agric. Sci., Univ. Florida, Gainesville, Florida.

MOSSE, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annual Rev. Phytopathol. 11: 171–196.

_____. 1976. The role of mycorrhiza in legume nutrition on marginal soils. Pp. 275–292 *in* J. M. Vincent, A. S. Whitney & J. Bose (editors), Exploiting the Legume–*Rhizobium* Symbiosis in Tropical Agriculture. College Tropical Agriculture Misc. Publ. 145. Univ. Hawaii, Honolulu, Hawaii.

_____. 1981. Vesicular-arbuscular mycorrhiza research for tropical agriculture. Hawaii Instit. Trop. Agric. & Human Resources, Univ. Hawaii, Res. Bull. 194: 1–82.

MUNNS, D. N. & B. MOSSE. 1980. Mineral nutrition of legume crops. Pp. 115–125 *in* R. J. Summerfield & A. H. Bunting (editors), Advances in Legume Science. Royal Botanic Gardens, Kew.

NELSON, C. E. & G. R. SAFIR. 1982. Increased drought resistance of mycorrhizal onion plants caused by improved phosphorus. Planta 154: 407–413.

NICOLSON, T. H. 1967. Vesicular-arbuscular mycorrhiza—a universal symbiosis. Sci. Prog., Oxford 55: 561–581.

OLIVER, A. J., S. E. SMITH, D. J. D. NICHOLAS & W. WALLACE. 1983. Activity of nitrate reductase in *Trifolium subterraneum:* effects of mycorrhizal infection and phosphate nutrition. New Phytol. 94: 63–79.

OLLIVIER, B., Y. BERTHEAU, H. G. DIEM & V. GIANINAZZI-PEARSON. 1983. Influence de la variété de *Vigna unguiculata* dans l'expression de trois associations endomycorhiziennes a'vésicules et arbuscules. Canad. J. Bot. 61: 354–358.

PANG, P. C. & E. A. PAUL. 1980. Effects of vesicular-arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans. Canad. J. Soil. Sci. 60: 241–250.

PAUL, E. A. & R. M. N. KUCEY. 1981. Carbon flow in plant microbial associations. Science 213: 473–474.

PLENCHETTE, C. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Pl. & Soil 70: 199–209.

POND, E. C., J. A. MENGE & W. M. JARRELL. 1984. IMproved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. Mycologia 76: 74–84.

POWELL, C. 1976. Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils. Nature 264: 436–438.

POWELL, C. L. 1977. Mycorrhizas in hill-country soils. II. Effect of several mycorrhizal fungi on clover growth in sterilized soils. New Zealand J. Agric. Res. 20: 59–62.

RANGELEY, A., M. J. DAFT & P. NEWBOULD. 1982. The inoculation of white clover with mycorrhizal fungi in unsterile hill soils. New Phytol. 92: 89–102.

RHODES, L. H. 1984. Applications for VA mycorrhizal fungi in crop production. Pp. 1–7 *in* J. J. Ferguson (editor), Application of Mycorrhizal Fungi in Crop Production. Instit. Food & Agric. Sci., Univ. Florida, Gainesville, Florida.

RICHARDS, B. N. 1974. Introduction to the Soil Ecosystem. Longman, London.

ROSS, J. P. 1972. Influence of Endogone mycorrhiza on Phytophthora rot of soybean. Phytopathology 62: 896–897.

SAFIR, G. 1968. The influence of vesicular-arbuscular mycorrhiza on resistance of onion to *Pyrenochaeta terrestris*. M. S. thesis. Univ. Illinois, Urbana, Illinois.

SALINAS, J. G., J. I. SANZ & E. SIEVERDING. 1985. Importance of VA mycorrhiza for phosphorus supply to tropical pasture plants in acid soils. Pl. & Soil 84: 347–360.

SCHENCK, N. C. 1981. Can mycorrhizae control root disease? Plant Dis. 65: 230–234.

_____. 1987. Vesicular-arbuscular mycorrhizal fungi and the control of fungal root diseases. Pp. 179–191 *in* I. Chet (editor), Innovative Approaches to Plant Disease Control. John Wiley & Sons, New York.

_____ & K. Hinson. 1973. Response of nodulating and nonnodulating soybeans to a species of Endogone mycorrhiza. Agron. J. 65: 849–850.

_____ & V. N. Schroder. 1974. Temperature response of Endogone mycorrhiza on soybean roots. Mycologia 66: 600–605.

Schönbeck, F. & H. W. Dehne. 1979. The influence of endotrophic mycorrhiza on plant diseases. 4. Fungal parasites on aerial plant parts, *Olpidium brassicae*, TMV. Z. Pflanzenkr. Pflanzensch. 86: 103–112.

Skipper, H. D. & G. W. Smith. 1979. Influence of soil pH on the soybean-endomycorrhiza symbiosis. Pl. & Soil 53: 559–563.

Smith, G. S., R. W. Roncadori & R. S. Hussey. 1986. Interaction of endomycorrhizal fungi, superphosphate and *Meloidogyne incognita* on cotton in microplot and field studies. J. Nematol. 18: 208–216.

Smith, S. E. 1982. Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. New Phytol. 90: 293–303.

_____ & M. J. Daft. 1977. Interactions between growth, phosphate content and N_2 fixation in mycorrhizal and non-mycorrhizal *Medicago sativa* (alfalfa). Austral. J. Pl. Physiol. 4: 403–413.

Snellgrove, R. C., W. E. Splittstoesser, D. P. Stribley & P. B. Tinker. 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. 92: 75–87.

Trinick, M. J. 1977. Vesicular-arbuscular infection and soil phosphorus utilization in *Lupinus* spp. New Phytol. 78: 297–304.

Tu, J. C. 1978a. Prevention of soybean root nodulation by tetracycline and its effect on soybean root rot caused by an alfalfa strain of *Fusarium oxysporum*. Phytopathology 68: 1303–1306.

_____. 1978b. Protection of soybean from severe *Phytophthora* root rot by *Rhizobium*. Physiol. Pl. Pathol. 12: 233–240.

Wick, R. L. & L. D. Moore. 1984. Histology of mycorrhizal and nonmycorrhizal *Ilex crenata* 'Helleri' challenged by *Thielaviopsis basicola*. Canad. J. Pl. Pathol. 6: 146–150.

Yost, R. S. & R. L. Fox. 1979. Contribution of mycorrhizae to P nutrition of crops growing on an oxisol. Agron. J. 71: 903–908.

Zambolim, L. & N. C. Schenck. 1983. Reduction of the effects of pathogenic, root-infecting fungi on soybean by the mycorrhizal fungus, *Glomus mosseae*. Phytopathology 73: 1402–1405.

_____ & _____. 1984. Effect of *Macrophomina, Rhizoctonia, Fusarium* and the mycorrhizal fungus *Glomus mosseae* on nodulated and non-nodulated soybeans. Fitopatol. Brasil. 9: 129–138.

SYSTEMATICS AND ECOLOGY OF ECTOMYCORRHIZAL LEGUMES

Ian J. Alexander[1]

Abstract

A critical review of published records shows that typical ectomycorrhizas are restricted to: i) Caesalpinioideae, specifically to Amherstieae and a few genera *(Afzelia, Intsia, Eperua)* of Detarieae, ii) Papilionoideae *(Aldina, Pericopsis, Swartzia)* with caesalpinioid affinities, or iii) Papilionoideae in the tribe Mirbelieae. The absence of ectomycorrhizas in the majority of Detarieae examined contradicts previous views. The legumes that form typical ectomycorrhizas are with few exceptions tropical trees. The majority are African and are well represented in the Guineo-Congolian and Sudano-Zambezian domains. Attention is drawn to potentially ectomycorrhizal legume genera in South America. Where nutrients, especially phosphorus, are in very low or uncertain supply, or where organic nitrogen accumulates, ectomycorrhizas may offer a competitive advantage over VA mycorrhizas. In certain situations competition between ectomycorrhizal fungi and soil saprophytes may slow litter decomposition, thereby helping to establish and maintain such conditions. Once a degree of dominance by ectomycorrhizal legumes has been established, it is reinforced by the fact that only individuals capable of forming ectomycorrhizas can be incorporated into the existing mycelial network. This may promote the development of low-diversity stands.

I. Introduction

Of the seven mycorrhizal types recognized by Harley & Smith (1983), only two, ectomycorrhizas and vesicular-arbuscular (VA) mycorrhizas, occur in Leguminosae. Most legumes form VA mycorrhizas; however, an important minority of woody taxa, distinguished by their systematic position and ecology, form ectomycorrhizas.

Ectomycorrhizas are the characteristic mycorrhizal association of woody perennial plants in temperate and boreal zones. The widespread assumption that they did not occur in the lowland tropics was first brought into question by reports of their presence on certain Caesalpinioideae in the lowland evergreen forest of the Congo (Peyronel & Fassi, 1957, 1960; Fassi & Fontana, 1961, 1962; Fassi, 1963). Ectomycorrhiza have subsequently been confirmed on Caesalpinioideae elsewhere in the tropics (see section III). In addition, it is now accepted that the Dipterocarpaceae are consistently ectomycorrhizal throughout their range and that a limited number of other woody tropical taxa, including members of Papilionoideae and possibly Mimosoideae, form ectomycorrhizas. We can conclude therefore, that although the overwhelming majority of tropical tree species habitually form VA mycorrhizas, as shown in numerous surveys, ectomycorrhizal trees form an important element of certain tropical woodlands and forest. Furthermore, the occurrence of ectomycorrhizas has a marked systematic basis.

This is particularly true with respect to Leguminosae. The apparent association of ectomycorrhiza with the non-nodulating Caesalpinioideae compared with the nodulating Mimosoideae and Papilionoideae has been widely commented upon. Malloch et al. (1980) and Polhill et al. (1981) go further and draw attention to the prevalence of ectomycorrhizas in tribes Detarieae and Amherstieae, linking this to the potential of trees in these tribes to occur gregariously and to form dense, often monospecific, stands, characteristically on infertile soils. In recent years new information on ectomycorrhizal occurrence has become available. I incorporate this information here with a critical

[1]Department of Plant and Soil Science, University of Aberdeen, Aberdeen, AB9 2UE, Scotland, U.K.

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review of the older records in an attempt to refine and extend current hypotheses about the systematics and ecology of ectomycorrhizas in the Leguminosae.

II. The Mycorrhizal Types

Ectomycorrhizas and VA mycorrhizas can be distinguished by their morphology and anatomy, and by the taxonomic position of the fungal partner.

The characteristic features of an ectomycorrhiza (Fig. 1) are the fungal sheath, which surrounds the host root, and the labyrinthine fungal tissue, which penetrates between the root epidermal cells to form the Hartig net. From the sheath surface hyphae or hyphal strands radiate out into the substrate. There is some debate as to whether those roots where the sheath, or more importantly the Hartig net, is patchy or absent can properly be termed ectomycorrhizas. In Caesalpinioideae that have been examined microscopically, sheath and net are well developed (but see *Eperua*, section III). This also applies to two Papilionoideae, *Aldina heterophylla* (Singer, 1978) and *Pericopsis angolensis* (Högberg & Piearce, 1986). However, in the ectomycorrhizas of Australian Mimosoideae and Papilionoideae described by Warcup (1980, 1985) and Kope & Warcup (1986), one or both of these features are often poorly developed.

The fungal partners in temperate ectomycorrhizas come from a well-documented list of ascomycete or basidiomycete genera, or from the single zygomycete genus *Endogone*. Where the fungal partners of legume ectomycorrhizas have been recorded, they normally conform to this pattern (Redhead, 1968; Singer & Araujo, 1979; Singer et al., 1983; Alexander & Watling, unpublished; Thoen & Ba, 1987). Again the Australian ectomycorrhizas described by Warcup (1980) stand apart in that the fungal partner in some cases has been ascribed (Warcup, 1985) to *Glomus*, a zygomycete genus thought previously to form only VA mycorrhizas.

VA mycorrhizas do not have a sheath or Hartig net. The fungus forms a loose weft in the substrate around the root and penetrates inter- and intracellularly throughout the host root cortex, typically forming vesicles and arbuscules (Fig. 2). These structures, common in herbaceous infections, are often absent in leguminous tree roots, and infection by a VA fungus results in the formation of distinctive intracellular hyphal coils (Fig. 3).

The fungi of VA mycorrhizas belong to the zygomycete genera *Glomus*, *Sclerocystis*, *Gigaspora*, *Acaulospora*, *Scutellospora*, and *Entrophospora*. As far as is known, these fungi display little host specificity. In this they contrast to ectomycorrhizal fungi, which show varying degrees of host specificity, at least at the ecological level (Harley, 1984).

VA mycorrhizas and ectomycorrhizas promote the growth of the host plant, particularly on infertile soils. The primary mechanisms are thought to be efficient exploitation of immobile nutrients by mycorrhizal mycelium and/or competitive nutrient capture. Little is known of the relative efficiency of the two mycorrhizal types in these regards, but the capacity for strand formation and for phosphate storage in the fungal sheath are assumed to confer special advantages on ectomycorrhizas. Ectomycorrhizas have also been reported to improve water uptake, drought resistance, disease resistance, and uptake of some forms of organic nitrogen and phosphorus. These points are discussed in more detail later in relation to the ecology of ectomycorrhizal legumes (section V).

A number of plant species form both VA mycorrhizas and ectomycorrhizas, sometimes on the root system of a single individual. This has been reported for the legumes *Eperua leucantha* and *E. purpurea* (St. John & Uhl, 1983), *Afzelia bella*, *Gilbertiodendron preussii*, and *Pellegriniodendron diphyllum* (Alexander, unpublished), *A. pachyloba* (Newbery et al., 1988), *G. dewevrei* (Peyronel & Fassi, 1957), *Intsia palembanica* (Norani, 1983, and pers. comm.), and *Acacia rigens* (McGee, 1986). However many leguminous trees that form VA mycorrhizas do not form ectomycorrhizas even when adequate fungal inoculum is present, e.g., when they are growing alongside closely related ectomycorrhizal taxa (Alexander, unpublished; Newbery et al., 1988). In the absence of any evidence for a high level of host/fungus specificity this can only indicate that within Leguminosae

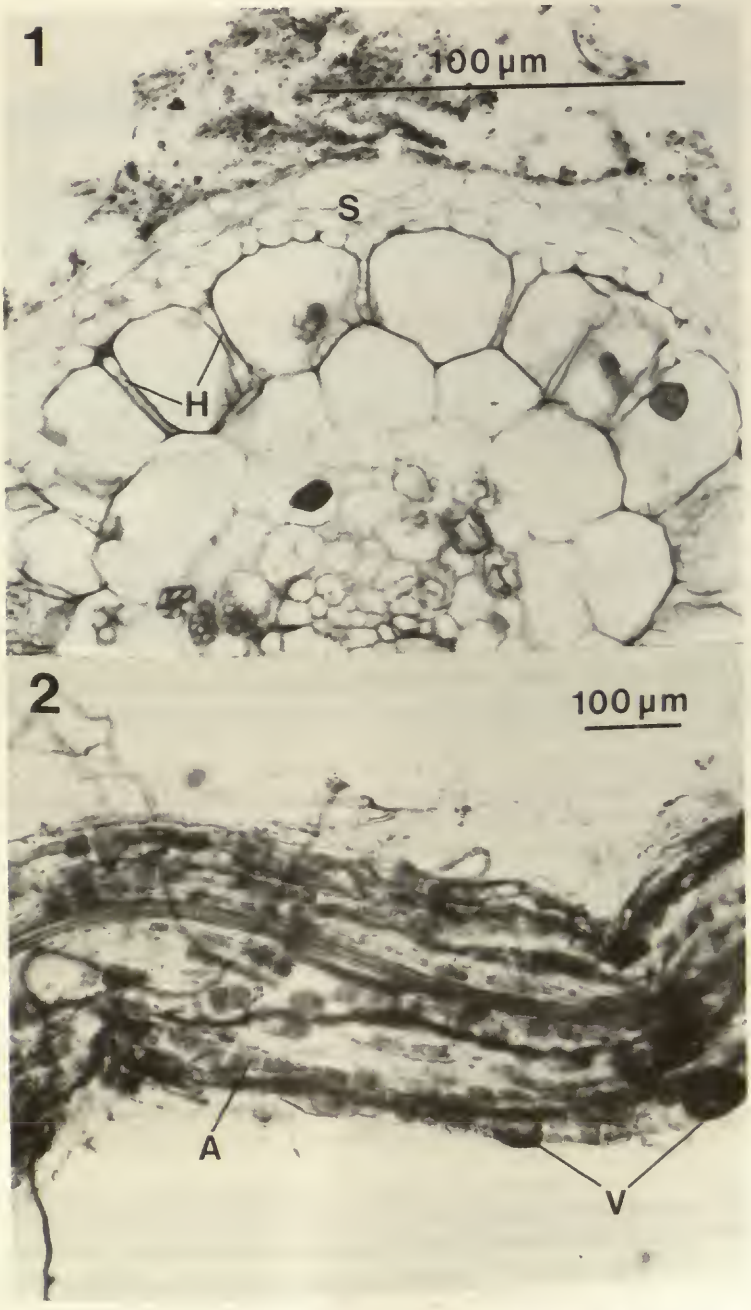

FIGURE 1. Transverse section of an ectomycorrhiza of *Afzelia bella* showing fungal sheath (S) and Hartig net (H).

FIGURE 2. Cleared and stained whole mount of a vesicular-arbuscular (VA) mycorrhiza of *Inga* sp. showing arbuscules (A) and vesicles (V).

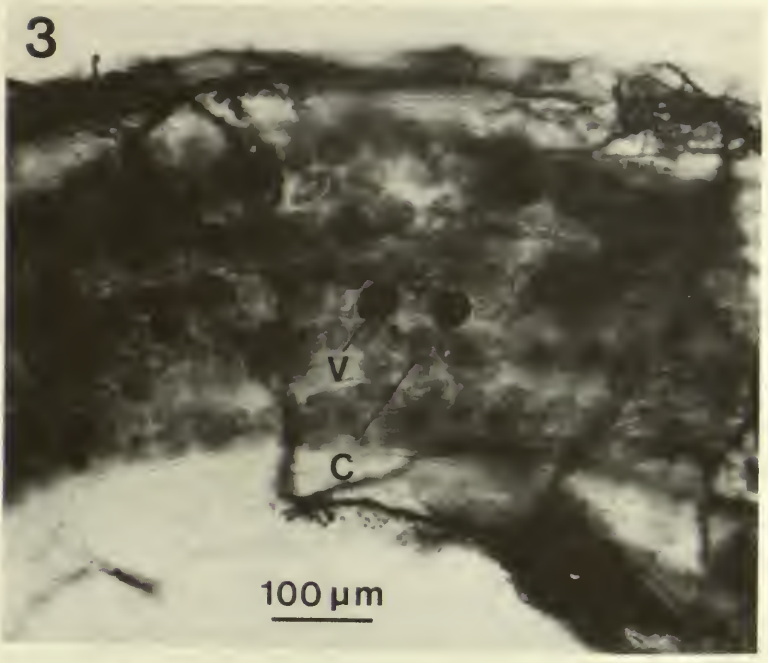

FIGURE 3. Cleared and stained whole mount of a vesicular-arbuscular (VA) mycorrhiza of *Daniellia pynaertii* showing hyphal coils (C) and vesicles (V).

some species are susceptible to ectomycorrhizal infection and some are not. Control of infection at this level apparently overrides control of infection related to environmental variables, such as soil fertility. The basis of this susceptibility, which is obviously much more restricted than susceptibility to VA mycorrhizal infection, is discussed further in section IIIA.

III. Records of Ectomycorrhizal Occurrence

In this section existing records of ectomycorrhizas in the Leguminosae are summarized at the generic level. Several introductory notes of caution are appropriate.

i. The absence of ectomycorrhizas from a single collection of a given species is a significant piece of information; however, it does not preclude the presence of ectomycorrhizas on the same species elsewhere.

ii. The ability to form ectomycorrhizas is assumed to be consistent within a genus (by analogy with temperate nonleguminous genera).

iii. An assumption of ectomycorrhizal status based on association between a tree and sporocarps of a fungus presumed by virtue of its taxonomic position to be ectomycorrhizal is unwise. Anatomical investigation of fine roots traced to the stem base of an identified host is the only sure method. Where my own unpublished records are cited this was the method employed.

iv. The problems of root tracing in tropical forest mean that many records are based on collections from seedlings or saplings or on small, possibly unrepresentative samples.

v. Certain existing records are misleading and are based on a misinterpretation of root or fungal

TABLE 1. Records of vesicular-arbuscular (VA) and ectomycorrhiza (EM) in the tribes of Caesalpinioideae. Specific weight of contributing genera[1] in brackets.

Tribe	Numbers of genera	Genera examined	Only recorded VA	Only recorded EM	VA and/or EM recorded
Caesalpinieae	47	13 (69%)	13 (69%)	0	0
Cassieae	20	5 (19%)	5 (19%)	0	0
Cercideae	5	2 (97%)	1 (2%)	0	1 (95%)
Detarieae	55	24 (64%)	21 (58%)	0	3 (6%)
Amherstieae	25	15 (64%)	1 (<1%)	12 (52%)	2 (11%)

[1]Number of species in genera examined/ total number of species in tribe × 100.

structures in poor preparations. Such records are referred to in the text. Inconsistent records unsupported by photomicrographs of mycorrhizas should be viewed with caution.

A. CAESALPINIOIDEAE

Records of the occurrence of mycorrhiza in the tribes of Caesalpinioideae (Cowan, 1981a) are given in Table 1.

1. Caesalpinieae

All members of this tribe for which I have found records of microscopic examination form VA mycorrhizas. These include the largest genus *Caesalpinia* (Maeda, 1954; St. John, 1980b; Norani, 1983; Alexander, unpublished), the nodule-forming genus *Erythrophleum* (Högberg & Piearce, 1986; Newbery et al., 1988), and the archaic relict genus *Gleditsia* (Maeda, 1954; Saleh-Rastin & Djavanshir, 1980). In the *Sclerolobium* group, considered by Malloch et al. (1980) to be of special interest because of its transitional position between Caesalpinieae and Detarieae, *Sclerolobium* (Alexander, unpublished) and *Tachigali* (Alexander, unpublished; Janos cit. Malloch et al., 1980) form VA mycorrhiza.

From the point of view of potential ectomycorrhizal formation the most interesting genera are *Mora* and *Dimorphandra*, species of which occur in low-diversity stands on white sand soils in, e.g., Guyana. *Mora excelsa* had VA mycorrhizas in Trinidad (Johnston, 1949). Norris (1969) described *M. excelsa* and *M. gonggrijpii*, from Guyana, as having roots "covered in short stubby outgrowths taken to be mycorrhizal rootlets." These rootlets were likened by Norris to those of *Eperua*, a genus since shown to form ectomycorrhizas (see below). Collections of *Dimorphandra exaltata* from Rio Doce, Brazil, were VA mycorrhizal (Alexander, unpublished). Definite conclusions on these two genera await further anatomical studies.

2. Cassieae

The data for Cassieae (Table 1) are biased by the inclusion of the partly herbaceous genera *Senna* and *Chamaecrista* for which no information is available. These genera are almost certainly VA mycorrhizal. If they are omitted from the calculations the specific weight of VA mycorrhizal genera recorded in Cassieae rises to 68%. *Cassia* has been found repeatedly to be VA mycorrhizal (Maeda, 1954; Redhead, 1960; Thomazini, 1974; Bakshi, 1974; Janos, 1980; Warcup, 1980; Herrera & Ferrer, 1980; Högberg, 1982; Norani, 1983). Thomazini also reported "ect-endomycorrhizas" on *Cassia cathartica*. This mycorrhizal type is thought to be restricted to Pinaceae (Harley & Smith,

1983). This and the absence of illustrations or anatomical descriptions, in addition to textual inconsistencies, in Thomazini's paper suggest that her report should be treated with caution (see also *Bauhinia* below).

3. Cercideae

The archaic genus *Cercis* forms VA mycorrhizas (Maeda, 1954). The largest genus *Bauhinia* has been found repeatedly to be VA mycorrhizal (Maeda, 1954; Shuja et al., 1971; St. John, 1980b; Malloch et al., 1980; Högberg, 1982; Norani, 1983). There are also two reports of ectomycorrhizas on *Bauhinia*. Thomazini (1974) described them as "well-developed" on *B. holophylla* from Brazilian "cerrado" vegetation, but there are reasons to view this report with caution. Tupas & Sajise (1976) reported ectomycorrhizas on *Piliostigma (= Bauhinia) malabaricum* from Phillipine savannah. Their photographs do not show ectomycorrhizas: secondary periderm tissue has been misinterpreted as a fungal sheath. I conclude that at present there is no good evidence that *Bauhinia* forms ectomycorrhizas.

4. Detarieae

Of the 55 genera in this tribe, 24 have been examined, and all, including the "core" genus *Cynometra*, have been found to form VA mycorrhizas (Table 1). *Afzelia*, *Intsia*, and *Eperua* also form ectomycorrhizas. Details of these records and their distribution within the groups of Detarieae are given in Table 2.

Afzelia bella (Redhead, 1960; Fassi & Fontana, 1962; Alexander, unpublished), *A. africana* (Jenik & Mensah, 1967; Redhead, 1968; Thoen & Ba, 1987), *A. quanzensis* (Högberg & Nylund, 1981), and *A. pachyloba* (Newbery et al., 1988) all form ectomycorrhizas. They have not been found on other Guineo-Congolian genera in the *Hymenostegia* group, i.e., *Hymenostegia*, *Talbotiella*, *Plagiosiphon*, and *Daniellia*, even where these grow alongside *Afzelia* (Newbery et al., 1988). They have, however, been found in Papua New Guinea on *Intsia bijuga*, (Alexander & Högberg, 1986; Horak, 1977), and in Malaysia on *Intsia palembanica* (Alexander & Norani, unpublished). The susceptibility of *Afzelia* and *Intsia* to ectomycorrhizal formation, in contrast to other genera in the *Hymenostegia* group, is interesting in view of the separate taxonomic position of these two genera indicated by their distinct pollen (Graham & Barker, 1981).

The documentation of ectomycorrhizas on the South American genus *Eperua* is much less complete, and detailed descriptions or illustrations are lacking. Singer & Araujo (1979) reported that species of *Eperua* were "probably" ectomycorrhizal in campanarina forest near Manaus, Brazil. On the other hand, St. John (1980a) found only VA mycorrhizas on *E. bijuga* in neighboring terra firme forest. Later St. John & Uhl (1983) reported "hyphae suggestive of a weakly developed ectomycorrhizal mantle" on *E. purpurea* and *E. leucantha* in caatinga at San Carlos de Río Negro, Venezuela. No other members of the *Brownea* group appear to have been examined.

5. Amherstieae

Fourteen of the 15 genera examined form ectomycorrhizas (Table 1), and two, *Pellegriniodendron* and *Gilbertiodendron*, also form VA mycorrhizas. Only *Tamarindus*, one of the isolated Afro-Asiatic genera, has been found to form only VA mycorrhizas.

Further details of the ectomycorrhizal records are given in Table 3. The genera in question are from Guineo-Congolian forest and/or the deciduous woodlands of south-central Africa. *Julbernardia* and *Brachystegia* are ectomycorrhizal in both situations. Information on the mycorrhizal status

TABLE 2. Records[1] of vesicular-arbuscular (VA) mycorrhiza or ectomycorrhiza (EM) in the tribe Detarieae.

Genera	VA	EM	Genera	VA	EM
Cynometra	10, 11		*Gossweilerodendron*	14	
Zenkerella	5		*Bathiaea*		
Lebruniodendron			*Apaloxylon*		
Umtiza			*Prioria*		
Maniltoa	1		*Agouardia*		
Scorodophloeus	5		*Stemonocoleus*		
Leonardoxa	10		*Hardwickia*		
Schotia			*Guibourtia*	7	
			Colophospermum	7	
Loesenera					
Hymenostegia	1, 10		*Goniorrhachis*	1	
Talbotiella	1		*Gilletiodendron*		
Plagiosiphon	10		*Tessmannia*		
Neochevalierodendron			*Baikiaea*	7	
Eurypetalum			*Hylodendron*	14	
Lysidice			*Sindora*	1	
Saraca	11		*Sindoropsis*		
Leucostegane			*Copaifera*	1	
Endertia			*Pseudosindora*		
Daniellia	10		*Detarium*		
Afzelia	1, 10	4, 6			
		9, 10	*Phyllocarpus*	1	
		14, 15	*Eperua*	16, 17	17, 18
Intsia	11	2, 8	*Thylacanthus*		
			Paloue		
Peltogyne	17		*Paloveopsis*		
Hymenaea	11		*Brachycylix*		
			Heterostemon		
Crudia			*Elizabetha*		
Oxystigma			*Brownea*		
Kingiodendron			*Browneopsis*		

[1]1, I.J. Alexander, unpublished observations; 2, Alexander & Högberg (1986); 3, Fassi & Fontana (1961); 4, Fassi & Fontana (1962); 5, Högberg (1982); 6, Högberg & Nylund (1981); 7, Högberg & Piearce (1986); 8, Horak (1977); 9, Jenick & Mensah (1967); 10, Newbery et al. (1988); 11, Norani (1983); 12, Peyronel & Fassi (1957); 13, Peyronel & Fassi (1960); 14, Redhead (1960); 15, Redhead (1968); 16, St. John (1980a); 17, St. John & Uhl (1983); 18, Singer & Araujo (1979).

of the South American genera *Dicymbe* and *Macrolobium*, which together contribute over 70 species to Amherstieae, and which occur in ecological situations comparable to those of Guineo-Congolian forest, is tantalizingly absent. Such information is central to any hypothesis on the timing of the development of the ectomycorrhizal habit in the group relative to the main periods of radiation (see Malloch et al., 1980).

6. Conclusions

It is clear that ectomycorrhizas are a consistent feature of Amherstieae, at least in Africa. Their absence from *Tamarindus* supports a separate line of development for that group. Otherwise, with respect to mycorrhizas, as with other features (Cowan & Polhill, 1981), Amherstieae appear to be uniform.

TABLE 3. Records[1] of vesicular-arbuscular (VA) mycorrhiza or ectomycorrhiza (EM) in the tribe Amherstieae.

Genera	VA	EM	Genera	VA	EM
Michelsonia			*Paramacrolobium*		4
Tetraberlinia		10	*Anthonotha*		1, 6
Microberlinia		10	*Anthonotha*		10, 13
Oddoniodendron			*Macrolobium*		
Pseudomacrolobium					
Englerodendron			*Humboldtia*		
Julbernardia		4, 7	*Tamarindus*	5, 11	
Julbernardia		10, 13	*Amherstia*		
Isoberlinia		7			
Berlinia		10	*Brachystegia*		4, 5
Dicymbe			*Brachystegia*		6, 15
Polystemonanthus			*Librevillea*		
			Monopetalanthus		4, 10
Gilbertiodendron	1, 12	1, 8, 12	*Aphanocalyx*		10
Pellegriniodendron	1	1	*Didelotia*		10
			Cryptosepalum		1

[1]References as in legend to Table 2.

Elsewhere in the subfamily the only unequivocal evidence for ectomycorrhizas is from *Afzelia, Intsia,* and possibly *Eperua,* and in these genera VA mycorrhizas are also present. The absence of ectomycorrhizas argues against the usefulness of a broad Detarieae–Amherstieae concept with respect to this important aspect of legume biology. General statements that both Amherstieae and Detarieae "regularly form ectomycorrhizae" (Malloch et al., 1980) or "probably always have ectomycorrhizae" (Polhill et al., 1981) are not supported by current evidence.

It is relevant to examine whether the distribution of other features within the subfamily can shed light on the factors controlling the susceptibility of certain groups to ectomycorrhizal infection. In this regard carbohydrate physiology and transport are of particular interest in view of the role of carbon in the establishment and maintenance of the symbiosis. Two features that have proved taxonomically interesting, in that they are concentrated in Detarieae and Amherstieae, are leaf phloem transfer cells (Watson & Gunning, 1981) and distinctive wall storage polysaccharides in cotyledons (Kooiman, 1960; Bailey, 1971; Smith, 1981). Both are well developed in all examined ectomycorrhizal genera; however, they are also found in many nonectomycorrhizal genera. The range and distinctive distribution of cell wall and other polysaccharides within Leguminosae (Bailey, 1971) is such that this area would repay further investigation, particularly in view of recent hypotheses on the role of the cell wall in the recognition process and morphogenesis of ectomycorrhizas (Harley & Smith, 1983; Harley, 1984; Lewis, 1986).

B. MIMOSOIDEAE

This subfamily is dominated by the three woody, largely tropical or subtropical genera, *Acacia, Inga,* and *Mimosa.* Ectomycorrhizas have been reported from *Acacia* and *Inga.* Many of the other genera are known to form VA mycorrhizas.

The main evidence for ectomycorrhizas in *Acacia* is from Warcup (1980, 1985). He identified ectomycorrhizas by the presence of a partial or entire sheath with or without a Hartig net. This wide definition has been criticized by Harley & Smith (1983). The twelve Australian *Acacia* species examined (Warcup, 1980) showed "looser associations of thin sheaths on roots" and only occasionally "typical-looking mycorrhizas." In pot experiments (Warcup, 1985), *Glomus tubiforme*

(Tandy) formed a sheath on roots of *A. retinodes* but apparently no Hartig net. McGee (1986), using Warcup's definition of ectomycorrhiza, recorded their presence on *A. rigens*. Sporocarps of *Hebeloma* (a genus containing known ectomycorrhizal species) (Horak, 1977), *Labyrinthomyces* (Kope & Warcup, 1986), and *Thelephora ramariodes* (Anonymous, 1983) have been recorded with *Acacia* and have been presumed to be ectomycorrhizal. *Thelephora ramariodes* has since been shown to behave more like a root parasite (Lee Su See, pers. comm.). All other investigators who have looked at *Acacia* roots (e.g., Maeda, 1954; Shuja et al., 1971; Lamont, 1982; Norani, 1983; Högberg & Piearce, 1986) have found only VA mycorrhizas. Whether or not the associations described by Warcup are usefully described as ectomycorrhizas, it appears on current evidence that they differ significantly in degree of development of sheath and net and in the nature of the fungal partner from the associations characteristic of Amherstieae.

Some confusion also surrounds the mycorrhizal status of *Inga*. *Inga laurina* (= *I. fagifolia*) and *I. vera* were reported to be ectomycorrhizal by Edmisten (1970) but recently a survey of plants from the same forest (Lodge, 1987) found only VA mycorrhizas, although basidiomycete hyphae were also present on root surfaces. Similarly, Singer (1975) included *Inga* among facultatively ectomycorrhizal genera, but in the present volume Singer concedes that this is by no means certain. Pegler & Fiard (1979) reported that *Lactarius* sporocarps were associated with *I. laurina*, but they did not examine the roots (Harley & Smith, 1983). My collections of *Inga* species and those of St. John (1980b) and Janos (1975) have contained only VA mycorrhizas. Further work to clarify the possible relationship between basidiomycetes and *Inga* roots is obviously required, but on present evidence typical ectomycorrhizas seem unlikely.

C. PAPILIONOIDEAE

Ectomycorrhizas have been reported from Swartzieae, Sophoreae, Mirbelieae, Bossiaeeae, Phaseoleae, Robinieae, and Vicieae. A very large number of both woody and herbaceous taxa form VA mycorrhizas.

1. Swartzieae

Singer (1978) considered *Aldina* to be obligately ectomycorrhizal and reported short roots "with mantle and Hartig net." Singer & Araujo (1979) detailed a range of basidiomycetes apparently symbiotic with *A. heterophylla* in campanarina. St. John & Uhl (1983) found that the only unequivocally ectomycorrhizal tree in terra firme or caatinga forest was "Macure," probably a species of *Aldina*.

Some species of *Swartzia*, the nearest relative of *Aldina*, were described by Singer & Araujo Aguiar (1979, 1986) as "probably" ectomycorrhizal in campanarina and "demonstrably" ectomycorrhizal in igapó. However, *S. reticulata* and another *Swartzia* species from terra firme forest proved to be nonmycorrhizal (St. John, 1980a), and *Swartzia myrtifolia* from Rio Doce, Brazil, was VA mycorrhizal (Alexander, unpublished).

The *Swartzieae* have in the past been regarded as caesalpinioid legumes. Their current position in Papilionoideae (Cowan, 1981b) is supported by, among other things, the proclivity of *Swartzia* to nodulate (Corby, 1981). A proclivity to form ectomycorrhizas, as shown by *Aldina*, argues, in contrast, for caesalpinioid affinities. *Aldina* has not been reported to nodulate (Allen & Allen, 1981).

A comprehensive survey of the mycorrhizal status of this tribe is required.

2. Sophoreae

Interest centers on the *Ormosia* group. *Pericopsis angolensis* was found with VA mycorrhizas at a miombo site in Tanzania (Högberg, 1982), but in a subsequent investigation (Högberg & Piearce,

TABLE 4. Australian genera of Papilionoideae forming ectomycorrhiza according to Warcup (1980).

Tribes	Genera
Mirbelieae	*Brachysema, Burtonia, Chorizema, Daviesia, Dillwynia, Eutaxia, Gompholobium, Jacksonia, Mirbelia, Oxylobium, Pultenaea, Viminaria*
Bossiaeeae	*Platylobium*
Phaseoleae	*Hardenbergia, Kennedia*

1986) there were no VA mycorrhizas, and a small proportion of the fine roots had ectomycorrhizas showing a well-developed sheath and Hartig net (Alexander & Högberg, 1986). The VA material but not the ectomycorrhizal material was also nodulated (Högberg & Piearce, 1986). There is an earlier, unsubstantiated, record of ectomycorrhizas on *Ormosia krugii* (Edmisten, 1970), but subsequent investigations at the same site have found only VA mycorrhizas (Lodge, 1987). All other Sophoreae for which records can be traced—*Amphimas, Baphia, Sophora, Maackia,* and *Cladrastis*—form VA mycorrhizas (Maeda, 1954; Högberg & Piearce, 1986; Alexander, unpublished).

Polhill (1981) described Sophoreae as "a tribe of convenience between Caesalpinioideae and the bulk of the Papilionoideae." The ability of some of its members to form ectomycorrhizas could, as with Swartzieae, be seen as an example of caesalpinioid affinities.

3. Mirbelieae, Bossiaeeae, Phaseoleae

Warcup (1980, 1985) has described similar associations to those of *Acacia* (see section IIIB) on a number of Australian papilionoid shrubs, mainly from the tribe Mirbelieae (Table 4). Here again a loose, thin, or partial sheath with or without a Hartig net is taken to constitute an ectomycorrhiza. However, Warcup stressed that "typical" mycorrhizas with complete sheaths and Hartig net occurred on *Pultenaea* and *Gompholobium,* and this has subsequently been confirmed by experimental syntheses (Kope & Warcup, 1986). *Pultenaea* also supports a typical ectomycorrhizal fungal flora (Watling & Gregory, 1988). Lamont (1982) considered *Dillwynia* and *Gastrolobium* (Mirbelieae) to be ectomycorrhizal.

4. Robinieae, Vicieae

There remain two further records of ectomycorrhizas in the Papilionoideae. *Robinia pseudoacacia* is normally reported to form VA mycorrhizas (e.g., Maeda, 1954), but Trappe (1962), citing Barsali (1922), listed *Robinia* as an ectomycorrhizal host of *Clathrus cancellatus.* This requires further investigation, particularly as the same "mycorrhizal-like mycelia" were seen on the roots of neighboring bamboos (see Kelley, 1950). *Clathrus* is not currently regarded as mycorrhizal.

The other record, also from Trappe (1962), in this instance citing Dominik (1957), is *Vicia sylvatica.* This is surprising, as typical ectomycorrhizas are not normally formed on herbaceous plants. In this case, however, the fungus was *Cenococcum geophilum,* which is known to have an exceedingly wide host range, including many normally VA mycorrhizal hosts (Trappe, 1964). The functional significance of such associations is unclear.

D. CONCLUSIONS

The distribution of ectomycorrhizas shows a broad correlation with current taxonomic groupings. Well-documented records of typical ectomycorrhizas, i.e., those with a more or less well-developed

sheath and Hartig net formed in association with basidiomycete or ascomycete fungi, are restricted to Caesalpinioideae, specifically to Amherstieae and a few genera *(Afzelia, Intsia,* possibly *Eperua)* of Detarieae, and to Papilionoideae *(Aldina, Pericopsis,* possibly *Swartzia)* with caesalpinioid affinities. Ectomycorrhizal formation therefore seems to be of real systematic value, as has been demonstrated previously for nodule formation (Corby, 1981) and rust infection (El-Gazzar, 1981). In addition to this more or less well-defined grouping, typical mycorrhizas obviously occur elsewhere in Papilionoideae in the shrubby tribe Mirbelieae based in southwestern Australia. This can be interpreted as a regional adaptation to low-phosphorus soils; whether it is of significance in legume phylogeny remains to be seen. The reports relating to *Acacia* appear to differ sufficiently in morphology and in the nature of the common mycobionts to be classed on current evidence with those other records, e.g., *Inga* and *Bauhinia,* which require further investigation. The possibility of ectomycorrhizal formation on a normally nonsusceptible host when growing in close proximity to habitually ectomycorrhizal neighbors should not be overlooked since VA mycorrhizal formation is known to be enhanced in similar circumstances (Hirrel et al., 1978).

IV. Biogeography

The legumes that form typical ectomycorrhizas are with few exceptions tropical trees, and the majority are African. They occur in Guineo-Congolian forest and the deciduous woodlands of East and South Central Africa. There are records from Ghana, Nigeria, Cameroon, Zaire, Tanzania, and Zambia. It can be assumed safely that the genera in question are ectomycorrhizal throughout their ranges on the continent. Thus west and north central African savannah woodlands also have an ectomycorrhizal element (e.g., *Isoberlinia doka, Berlinia grandiflora*). A full discussion of the biogeography and nutrition of tropical African legumes is given by Högberg (1986).

Ectomycorrhizas are likely to have been an important factor in the diversification and persistence of the relict genera in the Guineo-Congolian forest and the subsequent radiation into the savannah woodlands (Malloch et al., 1980; Polhill et al., 1981). At the time this radiation is thought to have taken place, in the Upper Tertiary, dipterocarps were present in East Africa (Ashton, 1983), and they still persist there *(Monotes, Marquesia).* Thus there has long been overlap in the distribution of the two most important groups of tropical ectomycorrhizal trees. However, ectomycorrhizal legumes are poorly represented in Asia (only *Afzelia* and *Intsia* are known). Raven & Polhill (1981) suggested that the penetration of legumes in general into Asia may have been limited by the prior existence of ecologically competitive groups. This appears to be the case with regard to the ectomycorrhizal niche, which in tropical Asia is dominated by dipterocarps.

The best-documented South American genera, *Aldina, Eperua,* and *Swartzia,* are components of Amazonian forest on infertile soils *(caatinga, campanarina, igapó).* A more thorough investigation of other potentially ectomycorrhizal legume genera in the continent is required before conclusions are drawn about their biogeography and evolutionary history.

The Australian group consists predominantly of shrubs and subshrubs from mediterranean or heathland ecosystems on infertile soils. It would be of great interest to know whether similar mycorrhizas occur in ecologically convergent groups in South Africa. The only records traced are for *Aspalathus* and *Rafnia* (Crotalarieae), which had VA mycorrhizas (Hoffman & Mitchell, 1986).

V. Ecology

Several recent reviews have sought to explain the ecology and distribution of ectomycorrhizal trees in the lowland tropics (Singer & Araujo, 1979; Malloch et al., 1980; Janos, 1983, 1985), and a number of overlapping hypotheses have emerged. These are summarized below and assessed with reference to legumes.

i. Ectomycorrhizas are of selective advantage on soils of very low fertility, e.g., white sand soils, because of efficient inorganic nutrient uptake and because of the ability of the mycobiont to utilize organic nitrogen and to decompose litter to some extent. By competing with saprotrophs, mycorrhizal fungi control decomposition rates and prevent nutrient loss through leaching. Surface accumulations of humus may further serve to exclude competitors.

It is true that there are striking assemblages of ectomycorrhizal legumes on poor soils, such as those in Zaire, Cameroon, or the campanarina of Amazonia. However, they are not restricted to such sites and have been recorded on a range of soil types. This is to be expected, for few tropical soils are likely to have phosphorus levels that would inhibit ectomycorrhizal formation. It is also entirely consistent with what is known of the behavior of ectomycorrhizal trees in temperate zones.

Although strands and sheaths are assumed to confer special advantages on ectomycorrhizas, the relative efficiency in nutrient uptake of ecto- and VA mycorrhizas has not been tested experimentally. The best support for increased competitiveness of ectomycorrhizas comes from Gartlan et al. (1986). They were able to show by the ordination of large-scale inventory data from Korup National Park, in southwestern Cameroon, that within this species-rich forest there was a marked floristic gradient correlated with the concentration of extractable phosphorus in the mineral soil. In particular there was a strong association between the large emergent ectomycorrhizal legumes *Microberlinia bisulcata, Tetraberlinia moreliana,* and *Didelotia africana* and areas where extractable P in the mineral soil in the wet season was $< 5\ \mu g\ g^{-1}$ oven dry soil. Subsequent surveys (Newbery et al., 1988) show that ectomycorrhizal legumes, of which there are about 18 species, make up ca. 50% of the basal area in groves up to 600 m across set in a matrix of ca. 165 other, VA mycorrhizal, tree species. Within these groves, in the dry season there is a marked accumulation of decomposing leaf litter intensely colonized by ectomycorrhizas.

The association between ectomycorrhizas and accumulation of dead organic matter at the soil surface in temperate regions is well known (Alexander, 1983), and Singer (Singer, 1984a, b; Singer & Araujo, 1979, 1986) reported on a similar phenomenon in Amazonian forest containing *Aldina, Eperua, Swartzia,* and other ectomycorrhizal trees. This association has encouraged the idea that ectomycorrhizas utilize organic nitrogen and/or decompose litter. There is growing evidence for the former (Alexander, 1983; Abu-Zinadah & Read, 1986) but still little for the latter (but see Trojanowski et al., 1984). However, the possibility that ectomycorrhizal fungi reduce decomposer activity by competition for mineral nutrients or by antibiosis (Gadgil & Gadgil, 1971, 1975) is of great interest. Singer & Araujo's (1979, 1986) comparisons of basidiomycete populations in campanarina, terra firme, igapó, and varzea forest supports the Gadgil hypothesis and they propose the sequence: oligotrophy, leading to ectomycorrhizal dominance, leading to organic matter accumulation, leading to nutrient conservation and closed nutrient cycles. However, it must be noted that where, in certain temperate forest, decomposer activity is not limited by nutrient availability, no significant Gadgil effect can be demonstrated (Harmer & Alexander, 1985). Furthermore, reduced decomposer populations and slow litter breakdown in ectomycorrhizal forest may be as much due to the poor substrate quality of the litter consequent upon nutrient stress (sclerophylly, high levels of secondary compounds) as to mycorrhizal suppression of saprophytes.

ii. Ectomycorrhizas are associated with single-species dominance or low species diversity. This results either from highly specific and effective fungal associations that favor competitive ability and promote abundance of one or a few host species (Janos, 1985) or from the presence of a wide range of mycobionts that maximize temporal and spatial exploitation of the site and promote stability in tree species composition (Malloch et al., 1980). Where host/fungus specificity exists the necessity for concomitant dispersal of host and fungal propagules promotes clumping.

Numerous reports cite ectomycorrhizal legumes as dominant components of vegetation (e.g., Fassi & Fontana, 1962, Singer & Araujo, 1979). In Table 5 some more recent data are presented. It is obvious that ectomycorrhizal legumes, singly or together, or with other nonlegume ectomycorrhizal taxa, can indeed dominate forest or woodland vegetation; but equally they continue to

TABLE 5. "Dominance" of ectomycorrhizal legumes in a number of forest types. BA = basal area; AGB = above biomass; AGV = aboveground volume; DBH = diameter at breast height.

Genera	Places	Dominance	References
Various Amherstieae + *Afzelia*[1]	Korup, Cameroon	10–55% BA	Newbery et al. (1988)
Afzelia, Anthonotha	Kade, Ghana	1% BA	Alexander & Swaine, unpublished
Gilbertiodendron	Bambesa, Zaire	76% stems > 32 cm DBH	Gerard (1960)
Tetraberlinia	Yoma-Gola, Liberia	55% stems > 20 cm DBH	Voerhoeve (1964)
Brachystegia, Julbernardia[2]	Morogoro, Tanzania	55–75% AGV	Högberg (1982)
Brachystegia, Julbernardia[2]	Misaka, Zambia	70% BA	Högberg & Piearce (1986)
? *Aldina*	San Carlos, Venezuela (terra firme)	4.5% AGB	St. John & Uhl (1983)
Eperua	San Carlos, Venezuela (caatinga)	19% AGB	St. John & Uhl (1983)

[1]Includes *Uapaca* (Euphorbiaceae).
[2]Includes *Uapaca, Monotes, Marquesia.*

form ectomycorrhizas even when they constitute only a small proportion of the stand. In this they resemble temperate ectomycorrhizal trees. Of the legumes said by Connell (1978) and Janzen (1974, 1981) to form low-diversity stands, *Eperua, Gilbertiodendron, Berlinia,* and *Brachystegia* are ectomycorrhizal, and *Peltogyne, Cynometra, Pentaclethra, Pterocarpus, Dimorphandra,* and *Mora* have only been recorded as VA mycorrhizal. There are no records for *Tessmannia, Prioria, Parkinsonia, Erythrina, Machaerium,* or *Dicymbe* (but see III A1 and III A5). Two additional species that form pure stands, *Talbotiella gentii* and *Colophospermum mopane,* are VA mycorrhizal (Alexander, unpublished; Högberg & Piearce, 1986). Thus, while ectomycorrhizal legumes may form low-diversity stands, not all low-diversity legume stands are ectomycorrhizal.

The relationship between host/fungus specificity and dominance of ectomycorrhizal trees has been much debated, and Janos (1985) has argued persuasively for it on theoretical grounds. So far the only experimental evidence is that of Smits (1983, 1985) for dipterocarps, but not enough detail is given to assess the validity of the claim. The wide range of ectomycorrhizal morphologies and basidiomycete sporocarps encountered in ectomycorrhizal legume forest and woodland argues against specificity, as does comparison with the temperate situation (Harley, 1984). In any case it is not necessary to invoke host/fungus specificity to explain how ectomycorrhizas contribute to dominance by their hosts (see below).

iii. Ectomycorrhizas are of selective advantage where there is a distinct dry season since their extensive network of mycelia and strands is an effective nutrient and water capture mechanism in an environment where nutrient and water availability is pulsed. The sheath serves as a storage mechanism for absorbed nutrient and protects against desiccation.

The African savannah woodlands conform to this pattern, and Högberg (Högberg & Nylund, 1981; Högberg, 1982; Högberg & Piearce, 1986) has demonstrated the importance of ectomycorrhizas in the phosphorus-poor miombo ecosystem.

The Australian ectomycorrhizal legumes may also fit in this category.

iv. Ectomycorrhizal species can be effective as gregarious colonizers of disturbed sites.

Only *Intsia bijuga* conforms to this hypothesis. The importance of wind-dispersed mycobiont spores in the process as proposed by Janos (1985) has not been tested.

v. Ectomycorrhizas impose a greater carbon drain on the host than VA mycorrhizas and are therefore limited to habitats where their advantages outweigh this "cost."

The carbon drain is difficult to measure, but theoretical argument (Janos, 1985) and "best estimates" (Harley, 1984) support the idea of a greater carbon movement to ectomycorrhizal than VA mycorrhizal fungi. It might be expected, however, that where trees (e.g., *Afzelia, Eperua*) have the alternative of forming VA or ectomycorrhizas, when the latter cease to be cost-effective their frequency on the root system will drop, rather than the tree cease to be competitive. With this in mind it seems more useful to argue for the positive attributes of ectomycorrhizas: only those trees capable of forming ectomycorrhizas can capitalize on the competitive advantages they offer on certain sites.

VI. Conclusions

Only certain legumes can form ectomycorrhizas and susceptibility has a phylogenetic basis. Legumes that can form ectomycorrhizas do so throughout their range. In certain situations, particularly where nutrients are in very low or uncertain supply, ectomycorrhizas offer a competitive advantage over VA mycorrhizas and numbers of ectomycorrhizal legumes will increase. Once a degree of dominance is established it is reinforced by the fact that only individuals capable of forming ectomycorrhizas can be incorporated into the existing mycelial network. There is now evidence that seedlings may receive carbon and nutrients from adult trees via mycorrhizal connections (Read & Finlay, 1985; Read & Francis, 1985).

Ectomycorrhizas may also be viewed as one of a suite of characters displayed by trees of nutrient-poor sites. Others are high nutrient use efficiency, sclerophylly, and the production of secondary compounds. These lead to slow litter decomposition, which may in turn be reinforced by competition between ectomycorrhizal fungi and saprophytes. Thus ectomycorrhizas may help establish and maintain conditions in which they are the most effective competitors for nutrients.

The idea that ectomycorrhizal trees form a "coterie" from which others are excluded is not new (Alexander, 1983; Harley, 1984). Certain legumes are in the coterie and this is reflected in their evolutionary success. The need now is for records (particularly from South America) to complete the systematic picture, and for experimental work on tropical legumes to test the hypotheses discussed above.

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References

Abu-Zinadah, R. A. & D. J. Read. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. New Phytol. 103: 481–514.

Alexander, I. J. 1983. The role of ectomycorrhizas in the nitrogen cycle. Pp. 69–93 *in* J. A. Lee, S. McNeill & I. H. Rorison (editors), Nitrogen as an ecological factor. Blackwell Scientific, Oxford.

——— & P. Högberg. 1986. Ectomycorrhizas of tropical angiosperm trees. New Phytol. 102: 541–549.

Allen, O. N. & E. K. Allen. 1981. The Leguminosae. A Source Book of Characteristics, Uses and Nodulation. Macmillan, London.

Anonymous. 1983. Mangium and other fast-growing acacias for the humid tropics. National Academy Press. Washington, D.C.

Ashton, P. S. 1983. Dipterocarpaceae. Pp. 237–552 *in* C. G. G. J. van Steenis (editor), Flora Malesiana 9. Martinus Nijhoff, The Hague.

Bailey, R. W. 1971. Polysaccharides in the Leguminosae. Pp. 503–541 *in* J. B. Harborne, D. Boulter & B. L. Turner (editors), Chemotaxonomy of the Leguminosae. Academic Press, London & New York.

Bakshi, B. K. 1974. Mycorrhiza and its role in forestry. Government of India Press, Dehra Dun.

Barsali, E. 1922. Contribuzione allo studio dei rapporti delle micorrize ecto-trofiche di alcune essenze arboree. Atti Soc. Tosc. Sci. Nat. Pisa Processi Verbali 31: 16–20.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science 199: 1302–1310.

Corby, H. D. L. 1981. Nodules. Pp. 657–670 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Cowan, R. S. 1981a. Caesalpinioideae. Pp. 57–64 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

———. 1981b. Swartzieae. Pp. 209–212 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & R. M. Polhill. 1981. Amherstieae. Pp. 135–142 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Dominik, T. 1957. Investigation of mycorrhizae in the beech forest near Baltykiem. Ekol. Polska Ser. A 5: 213–256.

Edmisten, J. 1970. Survey of mycorrhiza and nodules in the El Verde forest. Pp. F15–20 *in* H. T. Odum & R. F. Pigeon (editors), A Tropical Rain Forest, U.S.A. Atomic Energy Commission, Oak Ridge, Tennessee.

El-Gazzar, A. 1981. Systematic implication of susceptibility to *Uromyces* rusts in Leguminosae. Pp. 979–994 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Fassi, B. 1963. Die Vertailung der ektotrophen Mycorrhizen in der Streu und in der oberen Bodenschicht der *Gilbertiodendron devewrei*—(Caesalpiniaceae) Walder im Kongo. Pp. 297–302 *in* W. Rawald & G. Lyr (editors), Mykorrhiza. Gustav Fischer, Jena.

——— & A. Fontana. 1961. Le micorrize ectotrofiche di *Julbernardia serettii*, Caesalpiniaceae del Congo. Allionia 7: 131–157.

——— & ———. 1962. Micorrize ectotrofiche di *Brachystegia laurentii* e di alcune altre di Caesalpiniaceae minori del Congo. Allionia 8: 121–131.

Gadgil, R. L. & P. D. Gadgil. 1971. Mycorrhiza and litter decomposition. Nature 233: 133.

——— & ———. 1975. Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. New Zealand J. Forest Sci. 5: 33–41.

Gartlan, J. S., D. McNewberry, D. W. Thomas & P. G. Waterman. 1986. The influence of topography and soil phosphorus on the vegetation of Korup Forest Reserve, Cameroon. Vegetatio 65: 131–148.

Gerard, P. 1960. Etude ecologique de la foret dense a *Gilbertiodendron dewevrei* dans la region de l'Uele. Publ. Inst. Natl. Etude Agron. Congo Belge, Sér. Sci. 87: 1–159.

Graham, A. & G. Barker. 1981. Palynology and tribal classification in the Caesalpinioideae. Pp. 801–834 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Harley, J. L. 1984. The mycorrhizal associations. Pp. 148–186 *in* H. F. Linskens & J. Heslop-Harrison (editors), Encyclopedia of Plant Physiology 17, Interactions. Springer-Verlag, Berlin.

——— & S. E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press, London & New York.

Harmer, R. & I. J. Alexander. 1985. Effects of root exclusion on nitrogen transformations and decomposition processes in spruce humus. Pp. 267–277 *in* A. H. Fitter (editor), Ecological Interactions in Soil. Blackwell Scientific, Oxford.

Herrera, R. A. & R. L. Ferrer. 1980. Vesicular-arbuscular mycorrhiza in Cuba. Pp. 156–162 *in* P. Mikola (editor), Tropical Mycorrhiza Research. Clarendon Press, Oxford.

Hirrel, M. C., H. Mehravaran & J. W. Gerdemann. 1978. Vesicular-arbuscular mycorrhizae in the Chenopodiaceae and Cruciferae: do they occur? Canad. J. Bot. 15: 2813–2817.

Hoffman, M. T. & D. T. Mitchell. 1986. The root morphology of some legume spp. in the south-western

Cape and the relationship of vesicular-arbuscular mycorrhizas with dry mass and phosphorus content of *Acacia saligna* seedlings. S. African J. Bot. 52: 316–320.

Högberg, P. 1982. Mycorrhizal associations in some woodland and forest trees and shrubs in Tanzania. New Phytol. 92: 407–415.

_____. 1986. Soil nutrient availability, root symbioses and tree species composition in Tropical Africa: a review. J. Trop. Ecology 2: 359–372.

_____ & J. E. Nylund. 1981. Ectomycorrhizae in coastal miombo woodland of Tanzania. Pl. & Soil 63: 283–289.

_____ & G. D. Piearce. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation communities, and successional patterns. J. Ecol. 74: 775–785.

Horak, E. 1977. Biogeography of native ectomycorrhizal agarics in the southern hemisphere. P. 305 *in* Abstracts of the 2nd International Mycological Conference. Univ. of South Florida, Tampa.

Janos, D. P. 1975. Effects of vesicular-arbuscular mycorrhizae on lowland tropical rainforest trees. Pp. 437–446 *in* F. E. Sanders, B. Mosse & P. B. Tinker (editors), Endomycorrhizas. Academic Press, London.

_____. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rainforest plant growth. Ecology 61: 151–162.

_____. 1983. Tropical mycorrhizas, nutrient cycles and plant growth. Pp. 327–345 *in* S. L. Sutton, T. C. Whitmore & A. C. Chadwick (editors), Tropical Rain Forest: Ecology and Management. Blackwell Scientific, Oxford.

_____. 1985. Mycorrhizal fungi: agents or symptoms of tropical community composition? Pp. 98–103 *in* R. Molina (editor), Proceedings of the 6th North American Conference on Mycorrhizae. Oregon State Univ., Corvallis, Oregon.

Janzen, D. H. 1974. Tropical blackwater rivers, animals, and mast fruiting by the dipterocarpaceae. Biotropica 6: 69–103.

_____. 1981. The defenses of legumes against herbivores. Pp. 951–978 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Jenik, J. & K. O. A. Mensah. 1967. Root systems of tropical trees. 1. Ectotrophic mycorrhizae of *Afzelia africana* Gm. Preslia 39: 59–65.

Johnston, A. 1949. Vesicular-arbuscular mycorrhiza in Sea Island cotton and other tropical plants. Trop. Agric. (Trinidad) 26: 118–121.

Kelley, A. P. 1950. Mycotrophy in plants. Chronica Botanica Company, Waltham, Massachusetts.

Kooiman, P. 1960. On the occurrence of amyloids in seed plants. Acta Bot. Neerl. 9: 208–219.

Kope, H. H. & Warcup, J. H. 1986. Synthesised ectomycorrhizal associations of some Australian herbs and shrubs. New Phytol. 104: 591–599.

Lamont, B. 1982. Mechanisms for enhancing nutrient uptake in plants, with particular reference to Mediterranean South Africa and Western Australia. Bot. Rev. (Lancaster) 48: 597–689.

Lewis, D. H. 1986. Inter-relationships between carbon nutrition and morphogenesis in mycorrhizas. Pp. 85–100 *in* V. Gianinazzi-Pearson & S. Gianinazzi (editors), Physiological and Genetical Aspects of Mycorrhizae. INRA Press, Dijon.

Lodge, D. J. 1987. Resurvey of mycorrhizal associations in the El Verde rainforest, Puerto Rico. P. 127 *in* D. M. Sylvia, L. L. Hung & J. H. Graham (editors), Mycorrhizae in the next decade. Univ. of Florida, Gainesville, Florida.

Maeda, M. 1954. The meaning of mycorrhiza in regard to systematic botany. Kumamoto J. Sci. Ser. B, Biol. Geol. 3: 57–84.

Malloch, D. W., K. A. Pirozynski & P. H. Raven. 1980. Ecological and evolutionary significance of mycorrhizal symbiosis in vascular plants (a review). Proc. Natl. Acad. Sci. U.S.A. 77: 2113–2118.

McGee, P. 1986. Mycorrhizal associations of plant species in a semi-arid community. Austral. J. Bot. 34: 585–593.

Newbery, D. M., I. J. Alexander, D. W. Thomas & J. S. Gartlan. 1988. Ectomycorrhizal rain-forest legumes and soil, phosphorus in Korup National Park, Cameroon. New Phytol. 109: 433–450.

Norani, A. 1983. A preliminary survey on nodulation and VA mycorrhiza in legume roots. Malaysian Forester 46: 171–174.

Norris, D. O. 1969. Observations on the nodulation status of rainforest leguminous species in Amazonia and Guyana. Trop. Agric. (Trinidad) 46: 145–151.

Pegler, D. N. & J. P. Fiard. 1979. Taxonomy and ecology of *Lactarius* (Agaricales) in the Lesser Antilles. Kew Bull. 33: 601–628.

Peyronel, B. & B. Fassi. 1957. Micorrize ectotrofiche in una Caesalpiniaceae del Congo Belga. Atti Acad. Sci. Torino 91: 569–576.

_____ & _____. 1960. Nuovi casi di simbiosi ectomicorrizica in Leguminose della famiglia delle Casalpiniaceae. Atti Acad. Sci. Torino 94: 36–38.

Polhill, R. M. 1981. Sophoreae. Pp. 213–230 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____, P. H. Raven & C. H. Stirton. 1981. Evolution and Systematics. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. Part 1. Royal Botanic Gardens, Kew.

Raven, P. H. & R. M. Polhill. 1981. Biogeography of the Leguminosae. Pp. 27–34 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Read, D. J. & R. D. Finlay. 1985. Mycorrhizas and interplant transfer of nutrients. I. Ectomycorrhizas. P. 319 *in* R. Molina (editor), Proceedings of the 6th North American Conference on Mycorrhizae. Oregon State Univ., Corvallis, Oregon.

_____ & R. Francis. 1985. Mycorrhizas and interplant transfer of nutrients. II. Vesicular-arbuscular mycorrhizas. *In*: R. Molina (editor), Proceedings of the 6th North American Conference on Mycorrhizae, Oregon State Univ., Corvallis, Oregon.

Redhead, J. F. 1960. A study of mycorrhizal associations in some trees of western Nigeria. Diploma thesis. Univ. of Oxford.

_____. 1968. Mycorrhizal associations in some Nigerian forest trees. Trans. Brit. Mycol. Soc. 51: 377–387.

St. John, T. V. 1980a. A survey of mycorrhizal infection in an Amazonian rain forest. Acta Amazonica 10: 527–533.

_____. 1980b. Uma lista de espécies de plantas tropicais brasileiras naturalmente infectadas com micorriza vesicular-arbuscular. Acta Amazonica 10: 229–234.

_____ & Uhl. 1983. Mycorrhizae in the rain forest at San Carlos de Río Negro, Venezuela. Acta Sci. Venezolana 34: 233–237.

Saleh-Rastin, N. & K. Djavanshir. 1980. Mycorrhizal studies of Iranian forest trees. Pp. 154–155 *in* P. Mikola (editor), Tropical Mycorrhiza Research. Clarendon Press, Oxford.

Shuja N., U. Gilani & A. G. Khan. 1971. Mycorrhizal associations in some angiosperm trees around New University Campus, Lahore. Pakistan J. Forest. 20: 367–373.

Singer, R. 1975. The Agaricales in modern taxonomy, 3rd edition. Cramer, Vaduz.

_____. 1978. Notes on bolete taxonomy. II. Personia 9: 421–438.

_____. 1984a. Adaptation of higher fungi to varzea conditions. Amazonia 8: 311–319.

_____. 1984b. The role of fungi in Amazonian forests and in reforestation. Pp. 603–614 *in* H. Sioli (editor), The Amazon. Limnology and Landscape Ecology of a Mighty Tropical River and its Basin. Junk, Dortrecht.

_____ & I. J. G. Araujo. 1979. Litter decomposition and ectomycorrhiza in Amazonian forest. I. A comparison of litter decomposing and ectomycorrhizal basidiomycetes in latosol-terra-firme rain forest and white podzol campinarana. Acta Amazonica 9: 25–41.

_____ & I. Araujo Aguiar. 1986. Litter decomposition and ectomycorrhizal Basidiomycetes in an igapó forest. Pl. Syst. Evolution 153: 107–117.

_____, _____ & M. H. Ivory. 1983. The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially central Amazonia. Nova Hedwigia 77: 1–399.

Smith, D. L. 1981. Cotyledons of the Leguminosae. Pp. 927–940 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

Smits, W. Th. M. 1983. Dipterocarps and mycorrhiza. An ecological adaptation and a factor in forest regeneration. Fl. Males. Bull. 36: 3926–3937.

_____. 1985. Specificity of Dipterocarp mycorrhiza. P. 364 *in* R. Molina (editor), Proceedings of the 6th North American Conference on Mycorrhizae. Oregon State Univ., Corvallis, Oregon.

Thoen, D. & A. M. Ba. 1987. Observations on the fungi and the ectomycorrhizae of *Afzelia africana* and *Uapaca guineensis* in southern Senegal. P. 132 *in* D. M. Sylvia, L. L. Hung & J. H. Graham (editors), Mycorrhizae in the next decade. Univ. of Florida, Gainesville, Florida.

Thomazini, L. I. 1974. Mycorrhiza in plants of the 'Cerrado'. Pl. & Soil 41: 707–711.

Trappe, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28: 538–606.

_____. 1964. Mycorrhizal hosts and distribution of *Cenococcum graniforme*. Lloydia 27: 100–106.

Trojanowski, J., K. Haider & A. Hütterman. 1984. Decomposition of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. Arch. of Mikrobiol. 139: 202–206.

Tupas, G. L. & P. E. Sajise. 1976. Mycorrhizal associations in some savanna and reforestation trees. Kalikasan 5: 235–240.

Voorhoeve, l. A. G. 1964. Some notes on the tropical rainforest of the Yoma-Gola national forest near Bomi Hills, Liberia. Commonw. Forest. Rev. 43: 17–24.

Warcup, J. H. 1980. Ectomycorrhizal associations of Australian indigenous plants. New Phytol. 85: 531–535.

_____. 1985. Ectomycorrhiza formation by *Glomus tubiforme*. New Phytol. 99: 267–272.

Watling, R. & N. M. Gregory. 1988. Observations on the boletes of the Cooloola sand mass, Queensland and notes on their distribution in Australia. Part IIA—Introduction, keys and references. Proc. Roy. Soc. Queensland 97: 45–63.

Watson, L. & B. E. S Gunning. 1981. Leaf phloem transfer cells in Leguminosae. Pp. 717–722 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

REFLECTIONS ON MYCORRHIZAL RESEARCH

Rolf Singer[1]

ABSTRACT

Recently discovered or confirmed ectomycorrhizal hosts are enumerated and discussed with regard to legumes and Myrtaceae. Ectotroph-dominated forests in the neotropical lowlands have saprophytic fungi reduced to about one quarter of those present in anectotrophic forests. Ectotroph formers among Basidiomycetes are derived from root parasites. Only obligatory ectomycorrhiza formation has taxonomic significance in Basidiomycetes. Narrowly host-specialized ectomycorrhizal fungi exist, together with unspecialized species, in the tropics and in the temperate zone.

Mycologists specializing in mycorrhiza are divided into three groups: those that emphasize the physiological interaction between host and fungus; those that emphasize the anatomical side in order to determine which host enters into mycorrhizal relationships with which fungi; and the ecological group that examines the conditions under which mycorrhizal relationships arise and the phytosociological consequences of these relationships. The first group has long been and to a degree still is dominating the field, and this is natural because first mycorrhiza had to be defined. The second preoccupation holds special interest for the student of ectomycorrhiza and helps solve problems in the taxonomy of the host as well as the fungus. The third, attempting to explain the causal sequence of historical plant geography and phytosociology, is relatively young, but in ectomycorrhiza has shown some important interrelationships among soil, climate, and mycorrhization in forest communities, forest renewal and soil erosion, and related practical problems, particularly in the tropics. The following discussion refers only to ectomycorrhiza.

Whether a plant is ectomycorrhizal can be determined anatomically. If it has typical short roots with the Hartig net and mantle, the plant is ectomycorrhizal. Most host plants are only facultatively ectomycorrhizal. Under certain soil and climate conditions most typical host plants can develop to maturity even with poorly developed or no ectomycorrhiza. Ectomycorrhiza is a relatively recent (Pirozynski & Malloch, 1975) response to edaphic, climatic, or other kinds of adversity. Anatomists and taxonomists are interested in whether the host plant is capable of responding to certain growth substances by transforming rootlets into short roots capable of being penetrated by the ectotroph mycelium and establishing the exchange of nutrients, characteristic for the functioning of mutualistic symbiosis.

Except for the untangling of heterogeneous masses of intertwined roots in the tropics, it is relatively easy to dig out roots carefully from the upper layer of soil, photograph them, and preserve them in alcohol for further anatomical research. However, collectors and monographers, unless especially interested in mycorrhiza, usually do not dig out tree roots and describe them. Collectors have neither the time nor the routine to prepare the roots of all plants they collect, and monographers depend largely on herbarium material. Mycologists, wondering why a sudden change in the mycoflora takes place, find that forests of the world can be divided into ectotroph-dominated forests and anectotrophic forests where the ectotroph is far from dominant or is totally absent. Collectors of fungi of a group characterized by the frequent occurrence of ectotrophic mycelia search within a radius of about 10 m for short roots, follow these to the respective plant and identify it. It is therefore not surprising that beyond the well-known conifers and Fagales, *Tilia* (Tiliaceae), *Fraxinus* (Oleaceae) of the temperate regions, it is the mycologist who usually discovers and documents new and often unexpected host trees, including the following, identified and/or confirmed by

[1]Department of Botany, Field Museum of Natural History, Chicago, Illinois 60605, U.S.A.

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this author: Dipterocarpaceae, Ericaceae *(Arctostaphylus, Arbutus),* Gnetaceae, Leguminosae (Caesalpinioideae, Papilionoideae) but not all genera, Myrtaceae *(Eucalyptus, Leptospermum, Melaleuca),* Nyctaginaceae *(Neea, Pisonia, Torrubia),* Polygonaceae *(Polygonum viviparum, Coccoloba),* Rosaceae *(Dryas),* Sapindaceae *(Allophylus),* and Sapotaceae *(Glycoxylon).*

The list is not complete. It excludes some genera like *Uapaca* (Euphorbiaceae) *and Rhamnus* (Rhamnaceae) about which I have no personal experience, and all genera indicated do not form ectomycorrhiza in all species. Some species of *Psychotria* (Rubiaceae) occasionally form ectomycorrhiza, but the problem requires additional research.

The following comments consider two families in particular: Leguminosae and Myrtaceae. In the Myrtaceae *Eucalyptus* and *Leptospermum* belong in a subfamily, Leptospermoideae, which has been proposed as a separate family (Kausel, 1957). My experience shows that all Myrtoideae proper, tested thus far, do not produce ectomycorrhizal relationships in nature. Thus it appears that the separation of a family Leptospermaceae is justified on this basis also.

The legumes may or may not be in a similar situation. My experience has demonstrated that only genera formerly referred to Caesalpinioideae (two species in each of *Aldina* and *Swartzia*) are ectotroph formers (Singer, 1984b). Both genera belong to the tribe Swartzieae, which Cowan (1981) transferred to Papilionoideae. By contrast, their ectomycorrhizal associations suggest affinity with Caesalpinioideae. There is also evidence of *Eperua* (tribe Detarieae) being a neotropical ectotroph. Some former genera and several paleotropical genera of Caesalpinioideae evidently are capable of forming ectotrophs (Alexander & Högberg, 1986). I have believed for some time that *Inga* (Mimosoideae) forms ectomycorrhiza, as suggested by field observations of boletes in Bolivian coffee plantations where *Inga* is used as a shade tree. However, recent experience shows that *Gyrodon rompelii* and *G. adisianus* can develop amycorrhizically also.

In Amazonia, and judging from available data from the Paleotropics, the ectotroph forest of the lowland tropics is possible and is maintained only by way of direct cycling, that is, through the exchange of mineral nutrition and simple sugars in the mycorrhiza. Ectotroph dominance results where soil conditions are such that the root system of a nonmycorrhized plant is not capable of absorbing sufficient amounts of nitrogen and phosphorus in the form of soluble compounds from the soil, as in stands on deep white sand *(campina, campinarana)* or in stands inundated by black water *(igapó).* The extent and biomass, as well as some physiological properties of the ectomycorrhizal fungi, suppress the litter-decomposing fungi to such a degree that their number is reduced to about one quarter of the quantity present in an anectotrophic forest (Singer & Araujo, 1979, 1986). Consequently, the anectotrophic forest, with few or no ectotrophic mycelia, disposes of a surplus of litter decomposers and the soil is bare except for recently fallen leaves. By contrast, in the ectotroph forest, the litter layer grows and a deep raw humus layer is eventually formed. We have shown that the widespread Brazilian *terra firme* forest (Singer & Araujo, 1979) and the white water inundated forest are anectrotrophic forests (Singer, 1984a). This research has made it possible to confirm the forest types of Amazonia as outlined by Prance (1978), and to contribute to causative phytosociology, which explains the "why" behind the "which" in tropical forest communities in general (see also Lubini & Mandango, 1981).

Mycologists are interested in the specific roles and identities of fungi entering ectotrophic mycorrhiza. The situation becomes more complicated there than in the hosts. In order to be meaningful, the host–fungus relationship must be judged by several additional considerations: 1) to which category of ectomycorrhiza does the observed mycorrhiza belong? 2) which taxa of fungi are involved in mycorrhiza? 3) what degree of specialization does the respective species of fungi show in the field and under laboratory conditions?

We must strictly differentiate between: obligatory ectomycorrhiza; facultative ectomycorrhiza; cicatrizing mycorrhiza (Singer & Morello, 1960); and pseudomycorrhiza (not mutualistic).

The list of fungi acting as ectomycorrhizal fungi is meaningful taxonomically only if obligatory mycorrhiza is considered. The capability of forming obligatory ectomycorrhiza is currently an important character in Basidiomycete systematics. Even so, many families and genera, such as

Russulaceae, Boletaceae, and *Amanita* which are generally believed to be ectomycorrhizal, have some parasitic or saprophytic representatives. Examples include *Amanita nauseosa* (Singer, 1984a), *Lactarius igapoensis*, which is a root parasite on Apocynaceae and Tiliaceae (Singer, 1983), and *Xerocomus radicicola* (Singer, 1973). It is perhaps no coincidence that such exceptional species in generally ectomycorrhizal groups belong to the more primitive sections in their genera or families (Singer et al., 1983) and appear to be remnants of those root parasitic organisms from which the more derived, obligatory ectomycorrhizal taxa have developed. The species with widely diverse hosts are the adaptable forerunners of the most recent group of highly specialized species, such as many members of Boletineae (*Suillus*, *Gomphidius*, and *Leccinum*). Some of the species of this suborder have been enumerated (Singer & Araujo, 1986) as exclusively forming ectomycorrhiza with *Swartzia* in the periodically inundated *igapó*. Highly specialized and less- or nonspecialized species occur in the tropics as in other zones.

The ectotroph is, as Pirozynski (1983) expressed it, a "potential source of information concerning relationships of geographically significant taxa. Even preliminary fungal data can challenge botanical scenarios, assist in choosing between conflicting hypotheses or suggest alternatives." Any integrated view of the legume symbiosis will undoubtedly provide such a challenge.

References

Alexander, I. J. & P. Högberg. 1986. Ectomycorrhizas of tropical angiospermous trees. New Phytol. 102: 541–549.

Cowan, R. S. 1981. Tribe 1. Swartzieae. Pp. 209–212 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Kausel, E. 1957. Leptospermaceae. Angely, Cat. Estat. Gen. Bot. Fan. 28: 4.

Lubini, A. & A. Mandango. 1981. Etude physiosociologique et écologique des forêts à *Uapaca guineensis* . . . (Zaïre). Bull. Jard. Bot. Nat. Belg. 51: 231–254.

Pirozynski, K. A. 1983. Pacific mycogeography: an appraisal. Austral. J. Bot. Suppl. series 10: 137–159.

——— & D. W. Malloch. 1975. The origin of land plants. Biosystems 6: 153–164.

Prance, G. T. 1978. The origin and evolution of the Amazon flora. Interciencia 3: 207–222.

Singer, R. 1973. Notes on Bolete taxonomy. Persoonia 7: 313–320.

———.1983. Tropical Russulaceae II. *Lactarius* sect. *Panuoidei*. Nova Hedwigia, Beih. 77: 1–399.

———. 1984a. Adaptation of higher fungi to várzea conditions. Amazoniana 8: 311–319.

———. 1984b. The role of fungi in Amazonian forests and in reforestation. *In:* H. Sioli (editor), The Amazon 23: 603–614.

———& I. Araujo. 1979. Litter decomposition and ectomycorrhiza in Amazonian forests. Acta Amazonica 9: 25–41.

——— & ———. 1986. Litter decomposition and ectomycorrhizal basidiomycetes in an Igapó forest. Plant Systematics and Evolution 153: 107–117.

———, ———& K. H. Ivory. 1983. The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. Nova Hedwigia, Beih. 77: 1–399.

———& J. H. Morello. 1960. Ectotrophic forest tree mycorrhiza and forest communities. Ecology 41: 549–551.

A MODEL OF DAILY LEAF MOVEMENT IN RELATION TO THE RADIATION REGIME

Thomas J. Herbert[1]

Abstract

Plants from a wide range of taxonomic groups are capable of rapid reorientation of leaf surfaces in response to changes in light intensity, temperature, and plant water status. Since many excellent examples of nyctinastic and helionastic movements have been found in the Leguminosae, this family forms an important focus for understanding the physiology and ecology of diurnal leaf movement. Although movements have been described in considerable detail for many species of legumes and members of other plant families, and the physiological basis for these movements has been studied extensively, there has been little work on relationships between the geometry of leaf surface orientation and position, interception of solar radiation, and the physiological consequences of specific radiation regimes. This paper describes a conceptual framework, based on a distinction between scalar and vector stimuli, which is useful for analysis of such relationships. A simple computer model that permits computation of photosynthesis for different types of leaf movement is presented. This model is used to predict patterns of leaf surface movement as a function of the orientation of the direct solar beam and characteristics of the relationship between incident light flux and photosynthetic rate.

I. Introduction

Plants from many families are capable of rapid movement of leaves, leaflets, or pinnules (Darwin, 1881; Yin, 1938; Dubetz, 1969; Wien & Wallace, 1973; Wainwright, 1977; Schwartz & Koller, 1978; Shackel & Hall, 1979; Ehleringer & Forseth, 1980; Forseth & Ehleringer, 1980, 1982; Ehleringer, 1983; Herbert, 1984; Herbert & Larsen, 1985; Nilsen, 1985; Forseth & Teramura, 1986). Darwin (1881) identified several distinct types of leaf movement which include nyctinasty or sleep movement, characterized by vertical orientation of leaf surfaces at night, and helionasty, characterized by movement of leaf surfaces in response to incident solar radiation. Often, helionastic movements are characterized further as diahelionastic, for which leaf surfaces orient transversely to the direct solar beam, or parahelionastic, for which leaf surfaces orient parallel to the solar beam. These descriptions, however, may represent two extremes along a continuous gradient of behavioral response rather than distinct types of movement adapted for either maximization or minimization of absorption of solar radiation.

Generally, rapid and trophic diurnal leaf movements are the result of differential changes in the osmotic pressure of cells of the pulvinus, a specialized region at the base of the leaf, leaflet, or pinnule. Control of these changes is mediated by red or blue light absorbing photoreceptors located in the leaf or the pulvinus. While nyctinasty is usually associated with phytochrome, the red light absorbing pigment, helionasty, orientation to vectorial light, is associated with the blue-absorbing pigment (Gaillochet, 1967; Koller, 1981; Reed & Travis, 1984). Although the blue-absorbing pigment has not been identified, many studies have concentrated on the relationship of phytochrome to leaf movement. In one such study, Fondeville et al. (1966) found that regulation of *Mimosa pudica* leaflet closure is controlled by phytochrome. While most work suggests that the site for the phytochrome receptors is located in the pulvinus (Satter & Galston, 1971; Schremmpf et al., 1976; Wien & Wallace, 1973), the study by Schwartz & Koller (1978) on *Lavatera cretica* indicates that

[1]Department of Biology, University of Miami, Coral Gables, Florida 33124, U.S.A.

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the primary site for blue light absorbing photoreceptors associated with orientation to vectorial light sources is located in the leaf veins.

For *Albizia julibrissin,* Satter & Galston (1971) and Schremmpf et al. (1976) found that the osmotic pressure in the pulvinar motor cells is controlled by active transport of potassium and chloride across the cell membranes. Additionally, potassium and chloride concentrations have been shown to control turgor of pulvinar cells of *Samanea saman* (Satter et al., 1977) and potassium has been suggested to be involved in osmotic changes in other plants (Wainwright, 1977).

Although many investigators have examined the structure of the pulvinus and the control and mechanism of osmotic changes in the pulvinar cells (Funke, 1929; Satter & Galston, 1981; Wilkinson, 1983), few studies have examined cellular volume changes or osmotic and mechanical properties of the motor cells. Recently, Mayer et al. (1985) demonstrated that circadian leaf movement in *Phaseolus coccineus* is the result of an increase in length of motor cells on one side of the pulvinus and a decrease in length of the cells on the opposite side. During a cycle of circadian movement, the length of the peripheral extensor cells increases by about 20–30% and the length of the peripheral flexor cells decreases by about the same percentage. Furthermore, the length of the pulvinar motor cell is directly proportional to cell volume. Since the width of the motor cells does not change, work generated by osmotic pressure appears to be used efficiently for extension or flexion of the regions of the pulvinus.

These studies have contributed to significant progress toward an understanding of the details of the physical mechanism of rapid diurnal leaf movement. Additionally, the work of Ehleringer & Forseth (Ehleringer & Forseth, 1980; Forseth & Ehleringer, 1980, 1982; Ehleringer, 1983), in particular, has placed diurnal leaf movement in an ecological context by careful field studies and examination of the adaptive value of leaf movement. This research, however, has concentrated on the relationship of the physiology of individual leaves to ecology and has not considered the connections between helionasty and the geometrical relationships of plant leaves in detail. Not since Darwin (1881) proposed that all nastic movements were modifications upon periodic, "circumnutating" movements have helionastic and nyctinastic movements been placed into a single, theoretical framework based upon plant geometry. The purpose of the remainder of this paper is the development of a theoretical framework that will aid in understanding the physiological consequences and adaptive value of diurnal leaf movement.

II. Vectorial and Scalar Stimuli—Helionasty and Nyctinasty

Nyctinasty is characterized by inclination of leaf surfaces to a vertical position at dusk and reorientation to a horizontal position shortly before dawn. Usually there is a significant control of this cycle of movement by endogenous circadian rhythms; many nyctinastic plants continue to exhibit periodic leaf movement when kept in constant illumination or darkness (Satter & Galston, 1973).

Leaf or leaflet orientation may be characterized by the azimuth angle or compass direction into which the midrib points, α, the angle of inclination of the midrib above the horizontal, β, and the axial rotation or twisting angle, τ (Herbert, 1983a,b; Herbert & Larsen, 1985). I have observed that the leaflets of *Erythrina herbacea* (Herbert, 1984) and of many other genera and families, including *Lonchocarpus, Cassia,* and *Averrhoa,* move to a vertical position at dusk by decreasing the inclination angle β from positive values to negative values ($-60°$ to $-90°$). During this transition, axial rotation about the axis of the pulvinus decreases to near zero. Lasseigne (1980) made similar observations of the depression of leaf surfaces at dusk for 26 taxa of *Cassia,* and Darwin (1881) described a number of dicotyledons for which nyctinastic movements are similar to those described above except that leaves rise rather than fall into a vertical position at dusk. Monocotyledons, such as *Calathea lutea* (Herbert & Larsen, 1985), show similar nyctinastic orientation except that plant geometry is more often a limiting factor to the range of plant movements. Leaves of *C. lutea* fall

to a nearly horizontal position, unfold at dusk, then rise to a nearly vertical position and remain unfolded until shortly before dawn. From these observations, it appears that the characteristic geometrical feature of nyctinasty is the vertical orientation of leaf surfaces by elevation or axial rotation of leaflets or leaves about the pulvinar axis. Generally, the transition to and from nyctinasty appears to be characterized by changes in either β or τ but does not involve the elaborate movements of leaf surfaces associated with helionasty.

Darwin (1881) suggested that "in every country, and at all seasons, leaves must be exposed to nocturnal chills through heat loss by radiation, which might be in some degree injurious to them, and which they would escape by assuming a vertical position"; he thus proposed that vertical orientation of leaf surfaces reduced heat loss to the atmosphere. However, Bunning & Moser (1969) stated that "Darwin assumed that leaf movement protects the plant against chilling. This assumption cannot be considered satisfactory since it is not applicable to plants living under tropical conditions and leaf movements are most extreme in tropical plants. The present paper suggests that the adaptive value of leaf movement is . . . protection of the leaves . . . from moonlight." Bunning & Moser suggested that nyctinasty may have evolved as a mechanism to avoid exposure to moonlight and subsequent resetting of the circadian clock of the plant. In defense of Darwin's hypothesis, Enright (1982) argued that temperatures decrease enough in the tropics to result in deleterious physiological effects and demonstrated that while the leaf temperature of seedlings of *Phaseolus coccineus* was only about 1°C higher for vertically oriented leaves than for horizontally oriented leaves, this change was enough to affect growth rate significantly.

So, nyctinasty seems to be characterized by relatively simple movement to a nighttime vertical position, within the morphological constraints of plant structure. Present evidence suggests that the primary stimulus for such movement may be temperature, a one-dimensional, scalar quantity and that the adaptive value of this orientation is protection against lower temperatures than are optimal for physiological processes. Additionally, an adaptive value of vertical leaf orientation may be protection from illumination by moonlight, although there is no evidence that nyctinastic leaf movement vectorially tracks the moon's position so that the leaf surfaces are continuously reoriented to intercept a minimum amount of flux from the direct lunar beam.

Helionasty is generally characterized by a complex set of changes in leaf inclination, axial rotation or folding, and occasionally, azimuth. Darwin (1881) recognized the complexity of such movements when he described heliotropic movements as those which involve "circumnutating or bending successively in all directions."

Ehleringer & Forseth (1980) described changes in the azimuth of the steepest line on the leaf surface during solar tracking by the desert plants *Boerhaavia annulata* (Nyctaginaceae), *Lupinus arizonicus* (Leguminosae), and *Kallstroemia grandiflora* (Zygophyllaceae). For these species, it was noted that azimuth angles were usually within 15° of the solar azimuth, led the solar azimuth in the morning and lagged in the afternoon. Wainwright (1977) demonstrated similar behavior for *Lupinus arizonicus* with respect to the sun-tracking angle, the angle between the perpendicular to the leaf plane and the horizontal. One hour after dawn, the sun-tracking angle led the solar elevational angle by approximately 20°; at noon, the two angles were equal; and in the late afternoon the sun-tracking angle lagged the solar elevational angle by about 20°. Finally, for bean and sunflower plants, Shell et al. (1974) have shown that the phase angle between the leaf normal and the solar beam is positive in the morning and negative in the afternoon. For *Phaseolus vulgaris*, the phase angle indicated a lead of 38° in the morning, a lag of 13° near noon, and a lag of 44° in the afternoon.

In a study by me (Herbert, 1984), leaflets of the legume *Erythrina herbacea* were shown to rise rapidly at dawn to an average inclination angle, β, of about 25° and twist about the pulvinus to an average orientation with an axial rotation angle, τ, of about 30°. For this data, computer projections of a leaflet shape on a plane perpendicular to the sun demonstrated clearly that the leaflets faced the sun in the early morning and late afternoon, an orientation characteristic of diahelionasty, and away from the sun at noon, an orientation characteristic of parahelionasty (Herbert, 1984). However,

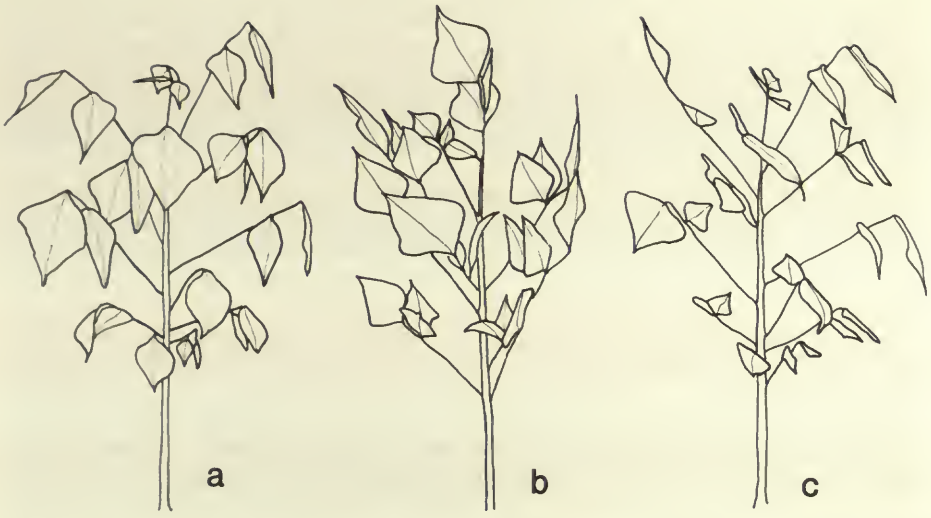

FIGURE 1. Orientation of leaflets of a specimen of *Erythrina poeppigiana* with a height of 1.2 m at: a) 21:30; b) 12:00; and c) 16:30 on 22 May 1984, Miami, Florida. (Redrawn from a photograph.)

analysis of the mean perpendicular vector to the leaflet surfaces shows that this vector leads the position of the sun in the morning and lags in the afternoon, and is oriented toward the sun at noon. Similar results have been obtained from studies by me on *Erythrina poeppigiana,* a tree native to tropical America. Analysis of photographs of a small specimen of *Erythrina poeppigiana* shows that leaflets are oriented in a nearly parallel array in the early morning or late afternoon and that the direction of the normal to the leaflet surfaces is about 30°–40° above the horizon as the sun is rising or setting (Fig. 1). Near noon, leaflet tips point toward the sun, reducing incidence of the direct solar beam upon the leaflet surface. However, the cosine of incidence, the cosine of the angle θ between a perpendicular to the leaflet surface and a vector to the sun, is about 0.21 for *E. herbacea* (Herbert, 1984) and 0.25 for *E. poeppigiana* at solar noon. A cosine of angle of incidence equal to 0.25 is equivalent to an average value of $\theta = 76°$ indicating that leaflet edges are not pointing directly at the sun, but are inclined with respect to the solar beam at an average angle of about 24°.

Similar results have been found on soybeans *(Glycine max)* by Kawashima (1969a, b). The mean position of perpendicular vectors to the leaf surfaces tracks the sun throughout the day, even though analysis of the cosine of angle of incidence would indicate that two distinct types of behavior might be taking place: diahelionasty in the early morning and late afternoon and parahelionasty near noon. The data of Kawashima (1969a, b) showed that directions of perpendicular vectors are more tightly clustered during the early morning and late afternoon than near noon (Fig. 2). In the morning these vectors lead the sun position, and in the evening they lag the sun position. Near noon, the perpendicular vectors form a circle about the sun position, with all but two data points characterized by an angle of incidence, θ, between 45° and 90°. However, in each case of continuous exposure to the direct solar beam, the leaf normals appear to be tracking the sun. When light intensity is low, as on cloudy days or when the plant is shaded from the direct solar beam, soybean leaves orient with perpendicular vectors tightly clustered. Near noon on a cloudy day, the leaf surfaces are horizontal: in the morning or afternoon, they are inclined so that the perpendicular vectors to the surfaces are directed at the region of the sky with highest flux.

The observations discussed above indicate that there may not be a useful distinction between diahelionasty and parahelionasty or, if such a distinction exists, it may not apply to consideration of helionasty during exposure to the direct solar beam. However, data on helionastic movements

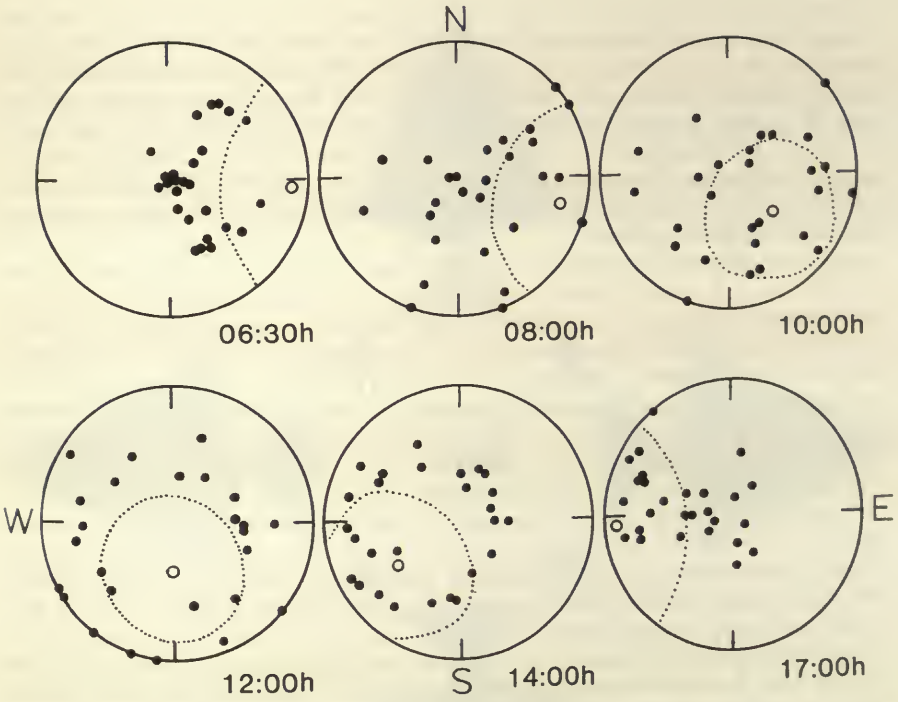

FIGURE 2. Daily changes in perpendicular vectors to the surfaces of soybean leaves on a sunny day (solid circles). The position of the sun is shown as an open circle, and a 45° cone about that position is shown as a dotted line. (Redrawn from Kawashima, 1969b.)

suggest that there is a strong relationship between the orientation of the direct solar beam and the perpendicular vector to a leaf surface. This relationship between leaf movement and a stimulus vector suggests that scalar quantities such as humidity, temperature, or scalar attributes of plant water status may not be the primary stimulus for leaf movement. Forseth & Ehleringer (1980, 1982) have shown a close relationship between leaf orientation and leaf water potential during paraheliotropic leaf movements of *Lupinus arizonicus*. However, changes in scalar stimuli such as soil moisture, humidity, and temperature may be the result of a vector stimulus such as the direct solar beam. Although more experimental work is needed to determine the relative importance of scalar stimuli for helionasty, the helionastic character of daytime leaf movement indicates that the relationship of the solar radiation regime to plant geometry forms a strong focus for understanding the significance of the various characteristics of diurnal leaf movement.

III. Computer Modeling of Light Interception and Self-shading

Models of light interception by individual leaves, isolated plants, or forests and plant stands are particularly useful tools for understanding the relationships between plant architecture and the physiological and adaptive characteristics of that architecture in relation to the radiation regime. For legumes, which commonly show complex diurnal leaf movements, modeling is particularly useful for isolation of those parameters of leaf orientation and movement that are most important for regulation of the radiation regime of the individual leaves and the resulting photosynthesis.

In many models of interception of radiation, light attenuation during penetration of a plant canopy is characterized by an attenuation coefficient for the direct solar beam or for scattered, diffuse illumination (Gutschick & Wiegel, 1984; Kuroiwa, 1970, 1978; Ross, 1981). These coefficients are derived as explicit formulae or an algorithm dependent upon the angles characterizing leaf orientation, the positions of leaves within the plant or plant stand, and the position of the sun. The flux incident upon a particular leaf is computed from the attenuation coefficient at the appropriate depth and the flux at the top of the canopy. Finally, if it is required, the relative photosynthetic rate may be calculated from the flux incident upon each leaf and integrated over all leaves to produce a measure of canopy photosynthesis.

Models that characterize the leaf canopy as a turbid medium suffer from some serious deficiencies (Myneni & Impens, 1985a, b; Myneni et al., 1986a, b). First, explicit mathematical models of the turbid medium are difficult to construct and use, and may lead to oversimplification. Second, they ignore the discrete nature of the individual leaf. While individual leaves may be considered in calculation of the attenuation coefficient, the use of such a coefficient to calculate photosynthesis on a particular leaf tacitly assumes that the surrounding leaves provide an isotropic environment. Such an oversimplification is likely to mask potentially interesting effects of leaf and canopy structure upon photosynthesis.

An alternative approach to the modeling of light interception and net photosynthesis of plants and plant stands is the calculation of light interception by computer simulation. Monte-Carlo simulation has been used by Oikawa (1977a, b) and Oikawa & Saeki (1977) for description of light microclimates in plant stands and the relationship of the incident light conditions to plant stand geometry and ray-tracing onto a faceted surface has been used by Geller & Nobel (1984, 1986) for calculation of light interception by cactus bodies.

In the present study, I present an exact method for calculation of interception of the direct solar beam, shading, and net photosynthesis from arrays of leaves. This method was implemented on a 512KB Macintosh computer using the language MacFORTH (Creative Solutions, Inc., Rockville, Maryland 20852, U.S.A.). Graphical constructions of each of 8 to 128 leaves in an array were defined using the region definition method in the Macintosh toolbox. These regions were then projected onto a plane perpendicular to the direction of the direct solar beam, sorted with respect to depth, and the unshaded areas computed using a fast routine written by me. The photosynthesis of each leaf surface was calculated from the unshaded area, the cosine of the angle of incidence of the direct solar beam with the leaf surface, and a relationship between photosynthetic rate (P) and incident light flux (I). For most computer experiments, this function was either a linear relationship, $P = aI$ (equation 1); or the familiar Michaelis-Menten relationship, $P = P_{max}I/(I+b)$, where P_{max} is the photosynthetic rate at saturating values of incident flux (equation 2).

A plant model chosen for study was an array of eight triangular leaves in two layers, each leaf having a base and length from pivot point to vertex of 100 units. The triangle is a generalized shape in the leaves of many phaseoloid legumes and the relative dimensions used in the model are typical of many such plants. The pivot point for elevational and rotational movement of each leaf was located 50 units from the axis of symmetry of the plant and the upper layer of leaves was rotated relative to the lower layer by an offset angle, θ_{offset}. Figure 3 shows a view of this model plant looking down the axis of symmetry of the plant. For the experiments described herein, the sun was always moved in zenith angle, ζ, from directly over the vertical axis ($\zeta = 0°$) to the horizon ($\zeta = 90°$) along the axis of two of the leaves in the lower layer, without any change in azimuth. While many other models could have been chosen for study, the simple model used demonstrates many of the most important aspects of interception of the direct solar beam by plant leaf arrays without the complexities associated with more elaborate models.

Initially the lower layer of leaves was fixed in a horizontal position and only the upper layer of leaves was permitted to move with respect to elevational angle, β, above the horizon and the axial rotation angle, τ, about the midrib. If the angle τ is fixed at zero for all leaves, β is permitted to vary for the upper layer of leaves, and a linear relationship is used for the relationship between flux

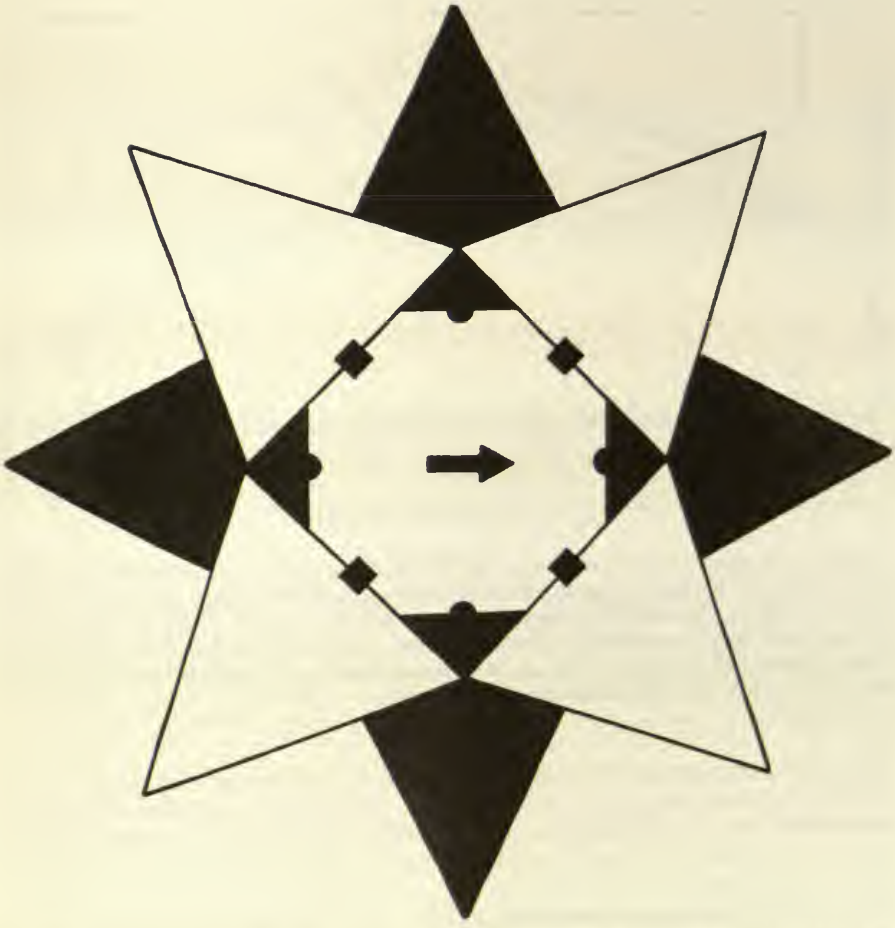

FIGURE 3. A scale drawing of the plant model used for computer simulations of light interception. The upper layer of leaves is indicated by unshaded triangles, the lower layer by shaded triangles, and the pivot point for each leaf by a square or circle. The arrow indicates the direction of sun movement from $\zeta = 0°$ (directly overhead) to $\zeta = 90°$ (on the horizon). The drawing represents a model with $\theta_{offset} = 45°$.

and photosynthetic rate. Results of the computer simulation demonstrate that the highest photosynthetic rates, P, are obtained when the sun is at zenith angles intermediate between 0° and 90°, and that generally, a horizontal orientation for the upper leaves results in higher values for P (Fig. 4). The value of ζ, for which there is maximum photosynthetic rate, depends strongly upon the size and spacing of the leaves, but the qualitative features of the relationships between P and the angles β, ζ do not.

If τ is varied and β is fixed at 0°, significantly greater values for photosynthetic rate are obtained at large values for the zenith angle of the sun, but otherwise the results of the simulation are similar to those for which only β is varied. However, if the relationship given by equation 2 is used to describe the photosynthetic rate as a function of incident flux, two important changes are observed. First, as shown in Figure 5, the photosynthetic rate is higher for many values of inclination of the leaf surface and is less dependent upon exact values of β, τ, or ζ. These phenomena are the direct

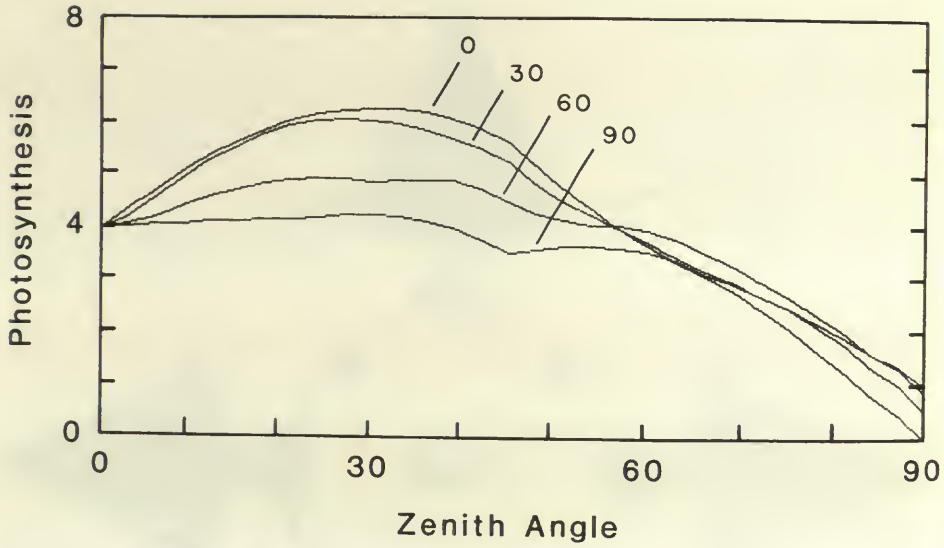

FIGURE 4. Photosynthetic rate as a function of zenith angle of the sun (°), ζ, for the computer simulation model. The inclination angle, β, was fixed at 0° for the lower layer of leaves, and at 0°, 30°, 60°, or 90° for the upper layer of leaves. The axial rotation angle, τ, was 0° for all leaves, $\theta_{offset} = 0°$, and a linear relationship between incident light flux and photosynthetic rate was assumed (equation 1). Photosynthetic rate is a dimensionless quantity which is computed as the ratio of the photosynthetic rate of the 8-leaf array to the photosynthetic rate of a single unshaded leaf illuminated perpendicularly.

result of the saturating property of the photosynthesis response function given by equation 2. More importantly, at solar zenith angles near 0°, the angle of leaf rotation, τ, which yields the highest photosynthetic rate is intermediate between 0° and 90°. (Similar results are produced when β is varied and τ is fixed at 0°.) Figure 6 summarizes this result for two models of leaf arrangement, $\theta_{offset} = 0°$, 90°, and values of b which give $P/P_{max} = 0.75, 0.95$ when the direct solar beam is incident perpendicularly upon a leaf surface.

Therefore, if the solar beam is directed down the axis of symmetry of the leaf array, maximum photosynthesis of the plant occurs when the leaf inclination angles are somewhere in the range of about 10° to 45° with respect to the axis of symmetry. Furthermore, as demonstrated in Figure 7, the variation in photosynthetic rate between leaves is a minimum for leaf inclination angles in the range for which photosynthetic rate is around its maximum.

The computer model described above was used with random values for the leaf orientation angles β, τ of each of the eight leaves. For each zenith angle, ζ, the combination of these 16 angles that yielded the maximum total photosynthetic rate was selected. From these combinations of angles, an average of the maximum inclination angle, σ, was calculated for the upper and lower layers of leaves. The angle σ was chosen as a measure of leaf inclination because it is a function of both β and τ and is the angle of elevation of the steepest line in the plane of leaf (Herbert, 1983a). Table 1 shows that the average inclination angle of the upper layer, as computed using circular statistics, was higher than that for the lower layer, and that the average photosynthesis was high and the standard deviation low compared with the models described previously for which leaves in the upper layer were constrained to incline at identical angles. Most significantly, the leaves moved as the zenith angle decreased such that the mean angle between the midrib of the leaf and the direct solar beam remained reasonably constant and within the range of angles predicted by previous experiments.

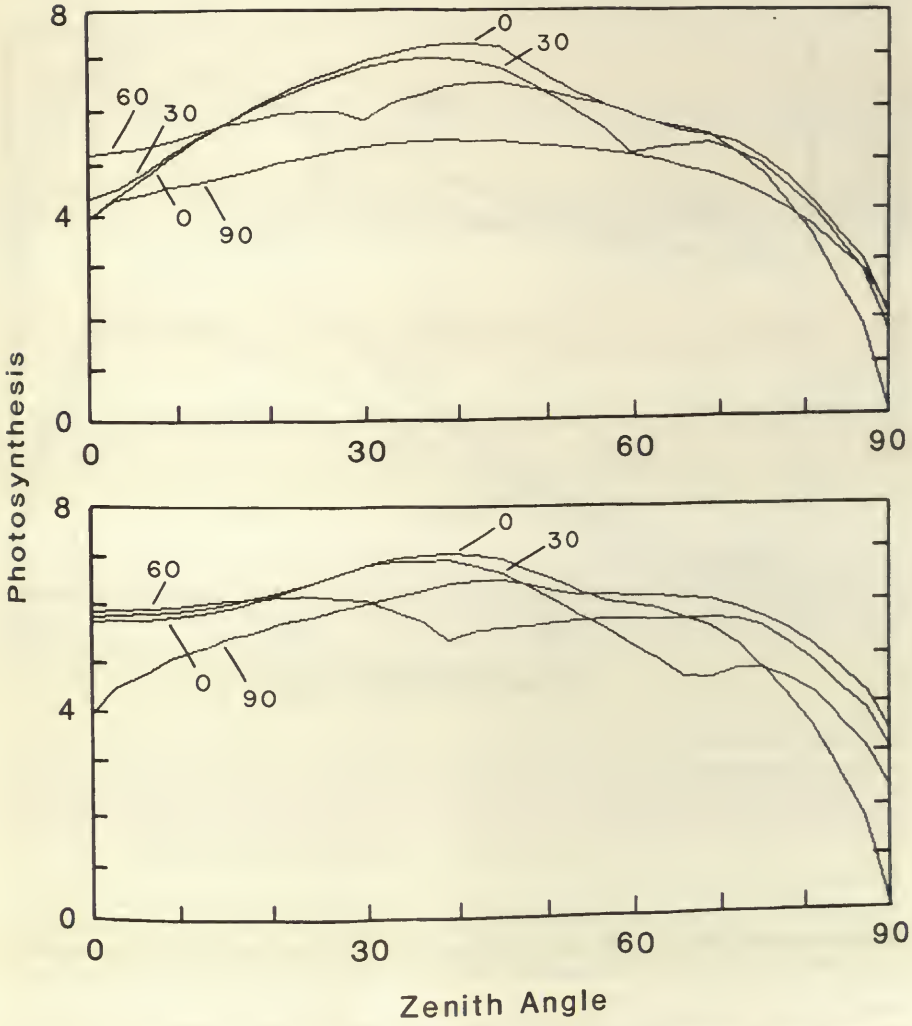

FIGURE 5. Photosynthetic rate as a function of zenith angle of the sun (°), ζ, for the computer simulation model: a) $\theta_{offset} = 0°$ (upper); b) $\theta_{offset} = 45°$ (lower). Units for photosynthetic rate are relative to that of a single leaf illuminated perpendicularly. The axial rotation angle, τ, was fixed at 0° for the lower layer of leaves, and at 0°, 30°, 60°, or 90° for the upper layer of leaves. The inclination angle, β, was 0° for all leaves, and a relationship between incident light flux and photosynthetic rate given by the Michaelis-Menten equation was assumed (equation 2) with $P = 0.75\ P_{max}$ when the incident flux was perpendicular to the leaf surface.

In most legumes, where leaves are able to move as the sun changes position, it might be expected that a suitable strategy for maximization of net photosynthesis may include some of the features of leaf orientation predicted by the computer model described above. This model does not consider the effects of either indirect radiation scattered from clouds, other leaf surfaces, nonphotosynthetic plant structures, or the ground. Additionally, it does not include the effect of penumbral radiation caused by the finite solid angle subtended by the sun's diameter (Horn, 1971). Since the fraction of scattered radiation is usually a small percentage of the total incident flux in the upper layers of

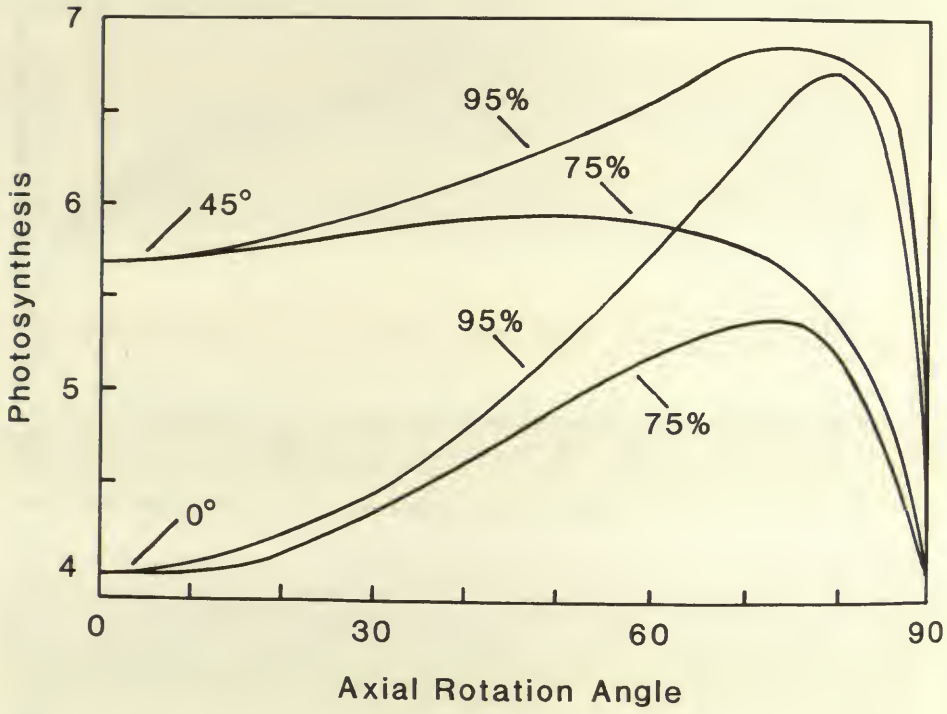

FIGURE 6. Photosynthetic rate as a function of axial rotation angle (°) for leaves in the upper layer, for the computer simulation models: θ_{offset} = 0°, 45°. The axial rotation angle, τ, was fixed at 0° for the lower layer of leaves, and the inclination angle, β = 0° for all leaves. A relationship between incident light flux and photosynthetic rate given by the Michaelis-Menten equation was assumed (equation 2) with P = 0.75 P_{max} (75%) or P = 0.95 P_{max} (95%) when the incident flux was perpendicular to the leaf surface.

TABLE 1. Photosynthetic rate, standard deviation of rate, and average leaf inclination as a function of zenith angle of the sun, ζ, for the computer simulation model with θ_{offset} = 0°. Angles β and τ were varied independently using 6,000 total combinations of angles for each of eight leaves to find a local maximum in total photosynthetic rate. This process was repeated for a total of five trials, each of which restricted the angular range searched to 0.1 of the search range for the previous trial. A realtionship between incident light flux and photosynthetic rate given by the Michaelis-Menten equation was assumed (equation 2) with P = 0.75 P_{max} when the incident flux was perpendicular to the leaf surface. Photosynthetic rate and standard deviation are dimensionless quantities, which are computed as the ratio of the photosynthetic rate or standard deviation of the 8-leaf array to the photosynthetic rate or standard deviation of a single unshaded leaf illuminated perpendicularly. Angles given in the table are in degrees.

ζ	Photosynthesis	Standard deviation	Average inclination angle	
			Upper layer	Lower layer
0	5.29	1.41	74.6	18.8
15	6.10	1.27	48.4	33.5
30	6.87	1.76	54.4	24.0
45	7.11	1.80	58.5	37.1
60	6.89	1.53	61.7	40.5
75	6.78	1.89	56.1	62.5
90	6.46	1.80	58.7	56.8

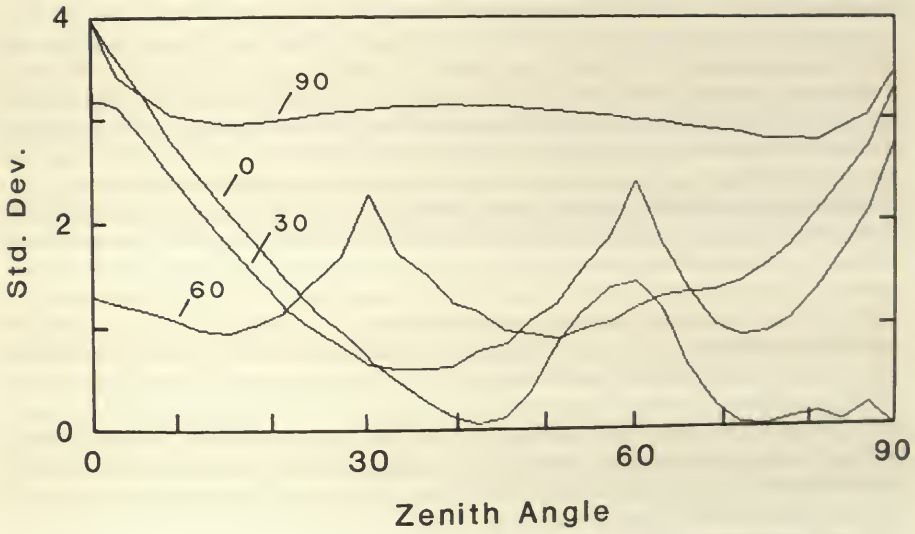

FIGURE 7. Standard deviation of photosynthetic rate as a function of zenith angle of the sun (°), ζ, for the computer simulation model: $\theta_{offset} = 0°$. All other parameters are identical to those described for Figure 5. Standard deviation of photosynthetic rate is a dimensionless quanity which is computed as the ratio of the standard deviation of the photosynthetic rate of the 8-leaf array to the standard deviation of the photosynthetic rate of a single unshaded leaf illuminated perpendicularly.

the plant canopy and is more isotropic than the direct beam, it may not play an important role in orientation of the top several layers of leaves. It has been suggested that the penumbra may be a significant component of incident flux for lower layers of leaves (Oker-Blom, 1984). Since most studies of helionasty have emphasized measurement of leaf orientation in the upper layers, the model described in this paper is likely to give realistic predictions and to avoid the most serious problems associated with scattered radiation and the penumbra. Future studies will concentrate on the effects of indirect radiation and on leaf orientation deep within a plant stand or forest canopy and build on the results of the present studies on the interaction of plant structure and the direct solar beam.

IV. Discussion

The computer simulation model described in this paper predicts many of the features of leaf surface orientation and helionasty observed for real plants. For a zenith angle $\zeta = 0°$, the model predicts that the lower layer of leaves should be oriented horizontally and the upper layer of leaves should be inclined at a shallow, but nonzero, angle with respect to the vertical.

For other low values of ζ, upper leaves should be inclined steeply with respect to the horizontal, forming a cone with respect to the direction of the direct solar beam. This prediction results from the following observations. Generally, the qualitative results of the model are independent of the relative orientations of the upper and lower layers of leaves and the orientation of the plant with respect to solar azimuth. Rotation and translation of the upper or lower layers with respect to each other affects the magnitude of total plant photosynthesis but does not influence strongly the value of inclination angle which results in maximum photosynthetic rate. Therefore, even if the solar

zenith angle is not zero, a set of leaf orientations for which the upper leaves were inclined at a steep, but nonzero angle with respect to the solar beam would result in high mean photosynthetic rate with low standard deviation.

Near noon, when the solar zenith angle ζ is small, the model predicts that the angle at which leaves are inclined with respect to the solar beam should be dependent primarily upon incident light flux and the photosynthetic characteristics of the leaf. From these results, we would expect only very weak dependence upon the shape of each leaf and its relative position in space.

In the early morning or late afternoon, when ζ is large, the inclination angles of leaves should be strongly dependent upon plant structure. For example, consider two leaves of the plant model shown in Figure 3, one placed directly above the other. Since the vertical spacing between the leaves in the upper and lower layers is equal to the length of the leaf from pivot point to vertex, there will be no shading of a leaf in the lower layer by a leaf directly above it if the leaf array is inclined at 45° with respect to the solar beam. If the array is inclined at a larger angle, the cosine of angle of incidence will decrease for each leaf; if the array is inclined at a smaller angle, shading will decrease the total leaf surface projected on a plane perpendicular to the solar beam. Obviously, changing the relative dimensions of leaf size and vertical spacing will have a pronounced effect on the optimal angle for inclination of the array of leaves. Additionally, the arrangement of leaf elements with respect to azimuth would be expected to influence strongly the optimum angle of orientation of leaves with respect to the direct solar beam for large values of ζ.

These results are supported by experimental observations which show that the positions of perpendicular vectors to leaf surfaces lead the sun in the early morning, lag the sun in the late afternoon, and orient in parallel arrays at times not close to solar noon. The computer model indicates that overall maximum values of photosynthetic rate occur when leaf inclination is zero and ζ is 45° or some other angle characteristic of leaf size and spacing. This geometry is identical to one in which the leaves are oriented in a parallel array that is inclined at an appropriate angle to the solar beam and is qualitatively the same as the geometry of leaf orientation in the early morning or late afternoon.

Neglecting the influence of shading upon light interception, I showed that light interception by plant leaves is highly dependent upon the azimuthal distribution and relative position of leaves or photosynthetic surface elements for large values of ζ and weakly dependent upon shape for small values of ζ (Herbert, 1983b). For large values of ζ, Ledent (1976, 1978) demonstrated that leaves with twisted or undulated surfaces intercept more of the direct solar beam than do flat leaves. These studies and the computer shading models appear to support the conclusion that leaf shape, structure, and orientation are important factors for maximum interception of the direct solar beam and photosynthesis, particularly when the sun is near the horizon.

The results of the Monte-Carlo simulations do not fit exactly many of the predictions described above. For $\zeta = 0°$, the optimal orientation for the lower layer of leaves should be a horizontal position, while the simulation predicts an inclination angle of about 23°. Similarly, the upper layer of leaves should be inclined at a somewhat steeper angle than predicted by the simulation. These and other discrepancies appear to be the result of a less than optimal search procedure used in the Monte-Carlo simulations. There appears to be a large set of combinations of β and τ that gives near to the global maximum for total plant photosynthetic rate. Thus the values found for β and τ vary from one search attempt to the next and map out a set of leaf orientations, all of which give a very high photosynthetic rate for the plant. Although more efficient search procedures will be used in future studies, the present results indicate that a large set of possible leaf orientations may result in nearly identical total plant photosynthetic rates. This result suggests that morphological constraints and other factors may play a significant role in determination of the exact details of leaf movement.

Additional support for the importance of morphological constraints in determination of the details of helionastic leaf movement is given by consideration of the relative effects of leaf inclination and axial rotation or folding upon light interception and total photosynthetic rate. The results of the computer model described here indicate that overall inclination of leaf surfaces with

respect to the horizontal is the most important factor in determination of total plant photosynthetic rate. Except for very large values of ζ, it appears that total photosynthesis is independent of whether this inclination is accomplished by varying β or τ. This observation is consistent with the results of the Monte-Carlo simulation. Monte-Carlo simulation using an inefficient search procedure with optimization for total plant photosynthesis usually results in prediction of an array of leaves that is both elevated (β) and rotated (τ), since there are many combinations of β and τ which give nearly equal total photosynthesis. Previously, I suggested that axial rotation or folding might be particularly important for maximization of light interception or photosynthesis when leaf shading was significant (Herbert, 1984; Herbert & Larsen, 1985). However, the results of the present study do not support this view.

Finally, leaf movements must be continuous in time. As the position of the sun changes, only a small subset of those combinations of elevational and rotational angles which gives maximum total plant photosynthesis will be found in the set of orientational angles that gives maximum photosynthesis for the next time increment. Clearly, continuity of motion places constraints on permissible values of β and τ for each leaf.

Richards (1966) noted that he and others have observed that "pulvini are an extremely common feature of the leaves of tropical trees and lianes," but offered no suggestion as to why this should be so. Near midday, tropical plants are exposed to high values of incident flux and solar elevation. The results of the computer model and the work of Kuroiwa (1970) suggest that, for high incident flux and small values of ζ, the upper layers of leaves in a plant canopy should incline at a very steep angle with respect to the horizontal. This orientation results in high total photosynthesis and protection of the uppermost leaves from water loss and heat stress.

McMillen & McClendon (1979) have shown that trees endemic to temperate regions are found to have sun leaves inclined at a significantly greater angle with respect to the horizontal than for shade leaves. However, of 11 species of trees studied, only two had average inclinations of sun leaves exceeding 36.8°. Tropical or subtropical plants such as those I studied (Herbert, 1984; Herbert & Larsen, 1985) commonly show average leaf elevations in full sun exceeding 80°. These data and results of the computer model support the idea that helionasty is adaptive for the extreme range of radiation flux and direction found in many tropical environments.

Some plants found in temperate regions exhibit helionastic leaf movement or have high leaf inclination angles. However, many of these plants are exposed to high solar flux or radiation scattered into low angles with respect to the vertical. McMillen & McClendon (1979) reported that the mean elevational angle for upper leaves of cottonwood exposed to full sun was 75.7°; I have observed that the mean leaf elevational angle of leaves of widely spaced cottonwood trees growing on highly reflective sand in White Sands National Monument, New Mexico, was not significantly different from 90°. Future studies on the comparative physiology and morphology of temperate plants that do and do not exhibit nastic leaf movements will be important for development of theories of adaptive value of nastic movements and the importance of these movements for plants growing in both tropical and temperate regions.

Clearly there is a close relationship between characteristics of the radiation regime of plants, the physiology of photosynthesis, and plant structure and ecology. Much more quantitative data is needed, particularly on plant physiology, leaf position and orientation, and ecology before the details of nastic leaf movements can be understood in an ecological and evolutionary context.

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References

Bunning, E. & I. Moser. 1969. Interference of moonlight with photoperiodic measurement of time by plants, and their adaptive reaction. Proc. Nat. Acad. Sci. U.S.A. 62: 1018–1022.

Darwin, C. R. 1881. The Power of Movement in Plants. D. Appleton & Co., New York.

Dubetz, S. 1969. An unusual photonastism induced by drought in *Phaseolus vulgaris*. Canad. J. Bot. 47: 1640–1641.

Ehleringer, J. R. 1983. Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. Oecologia (Berlin) 576: 107–112.

_____ & I. N. Forseth. 1980. Solar tracking by plants. Science 210: 1094–1098.

Enright, J. T. 1982. Sleep movements of leaves: in defense of Darwin's interpretation. Oecologia (Berlin) 54: 253–259.

Fondeville, J. C., H. A. Borthwick & S. B. Hendricks. 1966. Leaflet movements of *Mimosa pudica* L. indicative of phytochrome action. Planta (Berl.) 69: 357–364.

Forseth, I. N. & J. R. Ehleringer. 1980. Solar tracking response to drought in a desert annual. Oecologia (Berlin) 44: 159–163.

_____ & _____. 1982. Ecophysiology of two solar tracking desert winter annuals. Oecologia (Berlin) 54: 41–40.

_____ & A. H. Teramura. 1986. Kudzu leaf energy budget and calculated transpiration: the influence of leaflet orientation. Ecology 67: 564–571.

Funke, G. L. 1929. On the biology and anatomy of some tropical leaf joints. Ann. du Jardin Botanique de Buitenzorg 40: 45–73.

Gaillochet, J. 1967. Les mouvements foliolaires chez le *Cassia fasciculata* Mich.; influence de la glande pétiolaire sur le retour à l'état normal après excitation mécanique. Comptes Rendus de la Société de Biologie de Poitiers No. 3 (1976): 661–665.

Geller, G. N. & P. S. Nobel. 1984. Cactus ribs: influence on PAR interception and CO_2 uptake. Photosynthetica 18: 482–494.

_____ & _____. 1986. Branching patterns of columnar cacti: influences on PAR interception and CO_2 uptake. Amer. J. Bot. 73: 1193–1200.

Gutschick, V. P. & F. W. Wiegel. 1984. Radiation transfer in vegetative canopies and other layered media: rapidly solvable exact integral equation not requiring Fourier resolution. J. Quant. Spectrosc. Radiat. Transfer 31: 71–82.

Herbert, T. J. 1983a. The influence of axial rotation upon interception of solar radiation by plant leaves. J. Theor. Biol. 105: 603–618.

_____. 1983b. On the relationship between interception of direct solar radiation by cactus bodies and plant leaves. J. Theor. Biol. 105: 619–629.

_____. 1984. Axial rotation of *Erythrina herbacea* leaflets. Amer. J. Bot. 71: 76–79.

_____ & P. B. Larsen. 1985. Leaf movement in *Calathea lutea* (Marantaceae). Oecologia (Berlin) 67: 238–243.

Horn, H. S. 1971. The Adaptive Geometry of Trees. Princeton Univ. Press, Princeton, New Jersey.

Kawashima, R. 1969a. Studies on the leaf orientation-adjusting movement in soybean plants. I. The leaf orientation-adjusting movement and light intensity on leaf surface. Proc. Crop. Sci. Soc. Japan 38: 718–729.

_____. 1969b. Studies on the leaf orientation-adjusting movement in soybean plants. II. Fundamental pattern of the leaf orientation-adjusting movement and its significance for the dry matter production. Proc. Crop. Sci. Soc. Japan 38: 730–742.

Koller, D. 1981. Solar tracking (phototropism) in leaves of *Lavatera cretica* and *Malva parviflora*. Carnegie Inst. Wash. Year Book. 80: 72–75.

Kuroiwa, S. 1970. Total photosynthesis of a foliage in relation to inclination of leaves. Pp. 79–89 *in* I. Setlik (editor), Prediction and Measurement of Photosynthetic Productivity. Centre for Agricultural Publications and Documentation, Wageningen.

_____. 1978. Radiation environment and photosynthesis in plant stands with different foliage angles. Pp. 111–123 *in* M. Monsi & T. Saeki (editors), Ecophysiology of Photosynthetic Productivity. Tokyo Univ. Press, Tokyo.

Lasseigne, A. 1980. Studies in *Cassia* (Leguminosae-Caesalpinioideae) IV. The seedling, with special emphasis on sleep movement. Iselya 1: 187–199.

Ledent, J. F. 1976. Beam light interception by twisted leaf surfaces. Agric. Meterol. 17: 271–280.
_____. 1978. Beam light interception by leaves with undulating edges—a simulation of maize leaf sections. Agric. Meteorol. 19: 399–410.
Mayer, W. E., D. Flach, M. V. S. Raju, N. Starrach, & E. Wiech. 1985. Mechanics of circadian pulvini movements in *Phaseolus coccineus* L. Planta 163: 381–390.
McMillen, G. G. & J. H. McClendon. 1979. Leaf angle: an adaptive feature of sun and shade leaves. Bot. Gaz. (Crawfordsville) 140: 437–442.
Myneni, R. B. & I. Impens. 1985a. A procedural approach for studying the radiation regime of infinite and truncated foliage spaces. Part I. Theoretical considerations. Agric. Forest Meteorol. 33: 327–337.
_____ & _____. 1985b. A procedural approach for studying the radiation regime of infinite and truncated foliage spaces. Part II. Experimental results and discussion. Agric. Forest Meteorol. 34: 3–16.
_____, G. Asrar, E. T. Kanemasu, D. J. Lawlor & I. Impens. 1986a. Canopy architecture, irradiance distribution on leaf surfaces and consequent photosynthetic efficiencies in heterogeneous plant canopies. Part I. Theoretical considerations. Agric. Forest Meteorol. 37: 189–204.
_____, _____, G. W. Wall, E. T. Kanemasu & I. Impens. 1986b. Canopy architecture, irradiance distribution on leaf surfaces and consequent photosynthetic efficiencies in heterogeneous plant canopies. Part II. Results and discussion. Agric. Forest Meteorol. 37: 205–218.
Nilsen, E. T. 1985. Seasonal and diurnal leaf movements of *Rhododendron maximum* L. in contrasting irradiance environments. Oecologia (Berlin) 65: 296–302.
Oikawa, T. 1977a. Light regime in relation to plant population geometry. II. Light penetration in a square-planted population. Bot. Mag. (Tokyo) 90: 11–22.
_____. 1977b. Light regime in relation to plant population geometry. III. Ecological Implications of a square-planted population from the viewpoint of utilization efficiency of solar energy. Bot. Mag. (Tokyo) 90: 301–311.
_____ & T. Saeki. 1977. Light regime in relation to plant population geometry. I. A Monte Carlo simulation of light microclimates within a random distribution foliage. Bot. Mag. (Tokyo) 90: 1–10.
Oker-Blom, P. 1984. Penumbral effects of within-plant and between-plant shading on radiation distribution and leaf photosynthesis: A Monte-Carlo simulation. Photosynthetica 18: 522–528.
Reed, R. & R. L. Travis. 1984. Phototropic leaflet reorientation in alfalfa in response to vectorial light. Crop Sci. 24: 593–597.
Richards, P. W. 1966. The tropical rain forest. Cambridge Univ. Press, Cambridge. Pg. 89.
Ross, J. 1981. The Radiation Regime and Architecture of Plant Stands. W. Junk Publishers, The Hague.
Satter, R. L. & A. W. Galston. 1971. Potassium flux: a common feature of *Albizzia* leaflet movement controlled by phytochrome or endogenous rhythm. Science 174: 518–520.
_____ & _____. 1973. Leaf movements: rosetta stone of plant behaviour? Bioscience 23: 407–416.
_____ & _____. 1981. Mechanisms of control of leaf movements. Ann. Rev. Pl. Physiol. 32: 83–110.
_____, M. Schrempf, J. Chaudhri, & A. W. Galston. 1977. Phytochrome and circadian clocks in Samanea: rhythmic redistribution of potassium and chloride within the pulvinus during long dark periods. Pl. Physiol. 59: 231–235.
Schremmpf, M., R. L. Satter, & A. W. Galston. 1976. Potassium-linked chloride fluxes during rhythmic leaf movement of *Albizzia julibrissin*. Pl. Physiol. 58: 190–192.
Schwartz, A. & D. Koller. 1978. Phototropic response to vectorial light in leaves of *Lavatera cretica* L. Pl. Physiol. 61: 924–928.
Shackel, K. A. & A. E. Hall. 1979. Reversible leaflet movements in relation to drought adaptation of cowpeas, *Vigna unguicalata* (L.) Walp. Austral. J. Pl. Physiol. 6: 265–276.
Shell, G. S. G., A. R. G. Lang, & P. J. M. Sale. 1974. Quantitative measures of leaf orientation and heliotropic response in sunflower, bean, pepper and cucumber. Agric. Meteorol. 13: 25–37.
Wainwright, G. M. 1977. Sun-tracking and related leaf movements in a desert lupine *(Lupinus arizonicus)*. Amer. J. Bot. 64: 1032–1041.
Wien, H. C. & D. H. Wallace. 1973. Light-induced leaflet orientation in *Phaseolus vulgaris* L. Crop Sci. 13: 721–724.
Wilkinson, H. P. 1983. The anatomy of the pulvinus in various Mimosoideae in relation to function. Notes Jodrell Lab. 10: 1–10.
Yin, H. C. 1938. Diaphototropic movement of the leaves of *Malva neglecta*. Amer. J. Bot. 25: 1–6.

CYANOGENESIS IN THE LEGUMINOSAE

David S. Seigler[1], Bruce R. Maslin[2] and Eric E. Conn[3]

Abstract

Cyanogenesis has been reported from many members of the three subfamilies of the Leguminosae. Many of these reports need to be viewed critically and should be reconfirmed. To date, cyanogenic plants have been reported from four of the five tribes of the Mimosoideae and from all tribes of the Caesalpinioideae. The compounds responsible, however, have only been isolated from the genus *Acacia* (tribe Acacieae), where cyanogenic plants of the subg. *Acacia* contain proacacipetalin, epiproacacipetalin, heterodendrin, 3-hydroxyheterodendrin, proacaciberin, linamarin, and lotaustralin derived from the amino acids leucine, valine, and isoleucine. Cyanogenic plants from three Australian sections of subg. *Phyllodineae* contain prunasin and sambunigrin, derived from the aromatic amino acid phenylalanine. Cyanogenic members of the Papilionoideae have been reported from 18 tribes, but as above, many of these records need to be reevaluated. The ability to produce HCN upon hydrolysis has been reported from the Abreae, Adesmieae, Aeschynomeneae, Bossiaeeae, Cicereae, Coronilleae, Crotalarieae, Dalbergieae, Desmodieae, Galegeae, Genisteae, Indigofereae, Loteae, Phaseoleae, Robinieae, Sophoreae, Tephrosieae, Trifolieae, and Vicieae. The compounds responsible have been isolated only from a few of these tribes. Linamarin and lotaustralin have been found in many species of the Loteae and Trifolieae and probably occur in the Coronilleae. Vicianin (derived from phenylalanine) occurs in seeds of several *Vicia* species (Vicieae). At least one member of the Crotalarieae appears to contain prunasin. Cyanogenic compounds from the Galegeae (purportedly the parental group of these tribes according to Polhill (1981) have been reported, but their chemical nature is unclear. Members of the Indigofereae appear to contain prunasin or sambunigrin, whereas those of the Phaseoleae contain linamarin and lotaustralin. Cyanogenic species have been reported from the Tephrosieae, the basal tribe of this group, but no structures have been reported. One member of the Australian tribe Bossiaeeae contains the unusual cyanohydrin, *p*-glucosyloxymandelonitrile, and is alone in that characteristic in the Leguminosae. Holocalin and prunasin occur in the seeds and leaves respectively of *Holocalyx balansae* (Sophoreae). With the exception of proacacipetalin, epiproacacipetalin, proacaciberin, and 3-hydroxyheterodendrin from *Acacia*, no unique cyanogenic compounds are known to occur in the Leguminosae. Most of the tribes that contain cyanogenic compounds are relatively advanced. Because many families related to the Leguminosae (for example Rosaceae and Sapindaceae) frequently contain cyanogenic compounds, it is suggested that cyanogenesis either has been suppressed in the more primitive groups of legumes or has evolved independently in the various advanced groups. The former seems more probable. Cyanogenesis is most commonly associated with temperate legumes. Perhaps the accumulation of cyanogenic compounds in more advanced temperate groups is associated with ecological factors faced by the ancestors of these plants as they migrated from more tropical climates.

I. Introduction

What is cyanogenesis?

Numerous plants contain glycosides capable of releasing hydrogen cyanide (HCN) upon hydrolysis. The entire plant or any plant part may exhibit this property. These compounds usually co-occur with β-glycosidases that cleave sugars from the glycoside and hydroxynitrile lyases that decompose the resulting cyanohydrins. This property has been recorded in at least 2,050 plants from about 110 families (Gibbs, 1974; Hegnauer, 1977). The ability to produce and accumulate compounds of this type is found in all major plant groups but is especially common in several families (Saupe, 1981;

[1]Department of Plant Biology, University of Illinois, Urbana, Illinois 61801, U.S.A.
[2]Western Australian Herbarium, George Street, Kensington, Western Australia 6152, Australia.
[3]Department of Biochemistry and Biophysics, University of California, Davis, California 95616, U.S.A.

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Hegnauer, 1971, 1977). Most important of these are the Passifloraceae, Gramineae, Rosaceae, Sapindaceae, Ranunculaceae, Euphorbiaceae, Compositae, and Leguminosae.

METHODS FOR DETECTION OF CYANIDE

Most records of cyanogenesis are based on identification of the hydrolysis product, HCN, and in relatively few cases the parent compounds responsible have been isolated and characterized. This has been accomplished primarily with two relatively specific color tests. In both, paper strips are impregnated with test reagents and suspended over crushed or ground plant material. The endogenous enzymes usually are adequate to hydrolyze the cyanogenic glycosides, and gaseous hydrogen cyanide released reacts with the reagents on the test strips. In some instances the appropriate enzymes are added. The Guignard or Guignard-Mirande test uses sodium picrate as the active agent (Guignard, 1907; Mirande, 1909; Hegnauer, 1958, 1959). Chloroform or toluene are often added to the plant material to help break down plant tissue. This method is positive with material containing approximately 5 mg HCN per 100 g. The other test uses copper ethylacetoacetate and 4,4′-tetramethyldiaminodiphenylmethane (tetrabase) (Feigl & Anger, 1968; Tantisewie et al., 1969). The Feigl Anger test is more sensitive than the picrate method. Both tests are relatively simple and can be used in the field. The chemistry of these two tests as well as compounds that give false positive tests have been reviewed (Hegnauer, 1986).

Other methods employ the formation of Prussian blue for the detection of HCN (for example, Bruyning & van Haarst, 1899; Greshoff, 1906a, b; Guérin, 1928,1929; Gard, 1915). Many other methods for the qualitative identification of HCN have been reviewed (Seigler, 1977; Nahrstedt et al., 1981).

In other studies, quantitative methods were used. These include titration using silver nitrate with Liebig's iodine titration (or modifications thereof) (Dunstan & Henry, 1903, and many other references), the method described by Dragendorff (Bruyning & van Haarst, 1899), isolation by paper chromatography and subsequent hydrolysis and determination of cyanide (Butler, 1965), and the pyridine-pyrazalone method (Wokes & Willimott, 1951). In our laboratories, the modified Lambert method has proven dependable and sensitive (Lambert et al., 1975). A procedure for the quantitation of very small quantities of HCN has been published (Honig et al., 1983). In general, most of these methods are sufficiently sensitive to detect small amounts of cyanide, especially when concentrated by distillation.

The nuclear magnetic resonance (NMR) properties of the isolated compounds or derivatives (such as the trimethylsilyl ethers) are usually definitive with little chance of misidentification, especially with known compounds. These and other techniques for identification of cyanogenic compounds have been reviewed (Nahrstedt, 1981; Seigler, 1977).

Although R_f data from paper chromatography (PC) and from thin layer chromatography (TLC) are extremely useful, identification of compounds by these methods alone must be considered tentative.

In several studies, cyanogenic compounds (usually as their trimethylsilyl derivatives) have been separated and quantitatively measured by gas liquid chromatography (GLC). This method is fast and reliable in instances where the identity of the compounds is known (van Valen, 1979).

Are Cyanogenic Legumes a Threat to Health?

Although many commonly consumed legumes are reported to be cyanogenic, the amount of cyanide produced by most of these species is quite low and they pose little problem, if any, to the health of humans or domestic animals. Generally, only plants that accumulate more than 20 mg per

100 g fresh weight are considered dangerous (Kingsbury, 1964). For the amounts of cyanide produced, the reader should consult the original references.

The Literature

Although the literature for cyanogenesis in plants is voluminous and widely scattered, several major reviews have been published (Dunstan & Henry, 1906; Greshoff, 1906a, b, 1909; Puymaly, 1912; Rosenthaler, 1919, 1929; Robinson, 1930; Seifert, 1955; Dillemann, 1958; Eyjólfsson, 1970; Tapper & Reay, 1973; Gibbs, 1974; Seigler, 1977; Tson Sie Fat, 1979; Vennesland et al., 1981; Conn, 1981; Hegnauer, 1986). The application of information concerning cyanogenesis in plants to problems of systematics and phylogeny has been reviewed by Saupe (1981) and Hegnauer (1962–1973, 1971, 1977, 1986).

Problems in Interpretation of the Literature

Records of cyanogenesis in leguminous plants have been examined and reported in Table 1. These reports must be considered critically as various workers, methods, and materials have been involved.

Numerous records of cyanogenic plants were reported by Gibbs (1974). Many of these were attributed to "others," but the references to the works were not given. Records for which no additional reference could be found are indicated by an asterisk in Table 1. Gibb's results are reported in the usual way. Plants reported as cyanogenic by de Wildeman (1939) and Couperot (1913) were not considered reliable by Hegnauer (1958, 1959) and have not been cited in the present work. Where the presence of cyanide was determined by titration of distillates with silver nitrate alone (for example, Oke, 1964), the possibility of interfering substances cannot be excluded.

The results of cyanide tests reported by several workers (Herbert, 1922; Juliano, 1923, 1933; Juliano & Guerrero, 1935; Kalaw & Sacay, 1925; de Peralta, 1928; and Quisumbing, 1949) are open to question. In the modified procedure used in these studies, first suggested by Herbert (1922), the material to be tested was immersed in sodium picrate solution to which a little chloroform had been added. No information concerning possible interferences or confirmation of the efficacy of the method was presented. As the presence of cyanogenic compounds in many of the species tested have never been confirmed, the presence of cyanogenic glycosides in these plants is questionable. In the report of Kalaw & Sacay (1925), who worked under the direction of Professor Herbert, it is not clear what procedure was followed; they state "Guignard's method was used."

Several reports by Rosenthaler (1919, 1928, 1929) concerning cyanogenesis in seeds of *Vicia* species have never been confirmed by other workers (David Jones, pers. comm.).

Difficulties with Identification and Vouchering of Plant Materials

Despite the limitations of many of the chemical methods used, and with the exception of the references cited above, most positive tests probably do indicate the presence of hydrogen cyanide. A more serious problem concerns the identity of the plant materials studied. Few reports cite voucher specimens, and in many instances the plant materials were not identified by a person trained in plant taxonomy. While many common plants were undoubtedly correctly identified, there is no way to be certain and many records of cyanogenesis should be reconfirmed. There are also difficulties in ascertaining the currently accepted names for many of the plants studied.

Interpretation of Negative Reports

Although one can usually be certain that plants reported as positive for HCN do contain cyanogenic compounds (with some exceptions noted above), negative tests are more difficult to interpret.

TABLE 1. Reports of cyanogenic plants from the Leguminosae.

Species	Plant parts	References
	CAESALPINIOIDEAE	
	AMHERSTIEAE	
Tamarindus indica L.	lvs, frt, stbk, rtbk	Quisumbing (1949); Juliano & Guerrero (1935)
	CAESALPINIEAE	
Caesalpinia pulcherrima (L.) Sw.	lvs, st, rt	Quisumbing (1949); Herbert (1922)
Erythrophleum guineense G. Don.	lvs?	Sillans (1952)[qq]
Peltophorum inerme Naves ex Villar	stbk, rt, plt	Quisumbing (1949); Juliano & Guerrero (1935)
	CASSIEAE	
Cassia fistula L.	lvs, st, rt, sd	Quisumbing (1949); de Peralta (1928)
Chamaecrista flexuosa Green (syn.: *Cassia flexuosa* L.)	lvs	Kaplan et al. (1983)
Cassia sieberiana DC.	plt	Gibbs* (1974); Sillans (1952)[qq]
Cassia tora L.	lvs, bk, frt	Quisumbing (1949); Juliano (1933)
Senna alata Roxb. (syn.: *Cassia alata* L.)	lvs, st, rt	Quisumbing (1949); Herbert (1922)
Senna bacillaris (L.f.) Irwin & Barneby (syn.: *Cassia bacillaris* L.f.)	lvs, st, rt	Quisumbing (1949); Juliano (1933)
Senna bicapsularis (Harms) Irwin & Barneby (syn.: *Cassia bicapsularis* L.)	lvs	Kaplan et al. (1983)
Senna racemosa (P. Mill.) Irwin & Barneby (syn.: *Cassia racemosa* Mutis)	lvs	Kaplan et al. (1983)
Senna siamea (Lam.) Irwin & Barneby (syn.: *Cassia siamea* Lam.)	lvs, rt, st	Quisumbing (1949); Juliano & Guerrero (1935)
	CERCIDEAE	
Bauhinia carronii F. Muell	lvs	Webb (1949)
Bauhinia malabarica Roxb.	sd	Quisumbing (1949); Herbert (1922)
Bauhinia monandra Kurz.	lvs, stbk, rtbk, frt	Quisumbing (1949); Juliano & Guerrero (1935)
Bauhinia purpurea L.	sd?	Lucas & Sotelo (1984)
Bauhinia tomentosa L.	lvs, st, frt	Quisumbing (1949); Juliano (1933)
	DETARIEAE	
Intsia bijuga (Colebr.) Kuntze	lvs	Quisumbing (1949); Herbert (1922)
	MIMOSOIDEAE	
	ACACIEAE	
Acacia acatlensis Benth.	lvs	Seigler et al. (1978)
Acacia adsurgens Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia aroma Gill.	lvs	Seigler et al. (1979a); Seigler et al. (1979b); Seigler et al. (1983)[a]
Acacia atkinsiana Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia atramentaria Benth.	lvs	Seigler et al. (1979a); Seigler et al. (1979b); Seigler et al. (1983)[b]
Acacia beauverdiana Ewart & Scharman	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia berlandieri Benth.	lvs	Seigler, 1976b
Acacia bilimekii Macbride	lvs	Ebinger & Seigler, unpublished data
Acacia binervia (H.L. Wendl.) Macbr. (syn.: *A.glaucescens* Willd.)	lvs	Conn et al. (1985); Maslin et al. (1988)[g]; Finnemore & Cox (1928)[c]; Finnemore & Gledhill (1928a,b); Everist (1964); Hurst (1942)
Acacia blakei Pedley	lvs	Conn et al. (1985); Maslin et al. (1988)[g]

TABLE 1. Continued.

Species	Plant parts	References
Acacia burrowii Maiden	lvs	Everist (1964); Conn et al. (1985); Maslin et al. (1988)
Acacia californica Brandegee	lvs	Seigler et al. (1978); Conn et al. (1985); Maslin et al. (1988)[ll]
Acacia caroleae Pedley	lvs	Conn et al. (1978); Conn et al. (1985); Maslin et al. (1988)[11]
Acacia cheelii Blakeley	lvs	Everist (1964); Finnemore & Cox (1928)[c]; Finnemore & Gledhill (1928a, b); Hurst (1942); Maslin et al. (1988)[g]
Acacia chiapensis Saff.	lvs	Seigler et al. (1978)[b]; Rehr et al. (1973)
Acacia cochliacantha Humb. & Bonpl. ex. Willd.	lvs	Seigler et al. (1978)[b]; Seigler & Ebinger (1988)
Acacia conniana Maslin	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia constricta Benth.	lvs	Seigler et al. (1976)[b]
Acacia cunninghamii Hook.[e]	lvs	Secor et al. (1976)[c,d]; Finnemore & Gledhill (1928a,b); Hurst (1942); Conn et al. (1985)
Acacia curvinervia Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia deanii (R. Baker) Welch	lvs	Hurst (1942)
Acacia deanii subsp. *deanii*	lvs	Maslin et al. (1988)
Acacia deanii subsp. *paucijuga* (F. Muell. ex Wakef.) Tind.	lvs	Secor et al. (1976)[f]; Conn et al. (1985); Maslin et al. (1988)[ll]
Acacia dilatata Benth.	lvs	Gardner & Bennetts (1956)[pp]
Acacia diphylla Tind.	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia doratoxylon A. Cunn.	lvs	Finnemore & Gledhill (1928a,b); Everist (1964); Hurst (1942); Maslin et al. (1988)[g]
Acacia erioloba E. Mey. (syn.: *A. giraffae* Willd.)	lvs	Secor et al. (1976)[b]; Steyn & Rimington (1935); Steyn (1934b)
Acacia exilis Maslin	lvs	Conn et al. (1985); Maslin et al. (1988)[g,nn]
Acacia farnesiana	lvs, frt, sd	Secor et al. (1976); Herbert (1922); Seigler et al. (1979a)[a]
Acacia globulifera Saff.	lvs	Seigler et al. (1978); Seigler et al. (1983)[h]; Ebinger & Seigler (1987)
Acacia gonocarpa F. Muell.	lvs	Conn et al. (1985); Maslin et al. (1988)
Acacia gracillima Tind.	lvs	Conn et al. (1985); Maslin, Dunn & Conn (in prep.)[g,oo]
Acacia granitica Maiden	lvs	Conn et al. (1985); Maslin, Dunn & Conn (in prep.)[g]
Acacia greggii A. Gray[i]	lvs	Schmutz et al. (1968); Kingsbury (1964); Arizona Ag. Exp. Stat. (1934)
Acacia hebeclada DC.[b] (syn.: *Acacia stolonifera* Burch.)	lvs, frt, sd	Ettlinger et al. (1977); Steyn & Rimington (1935); Steyn (1934b); Rimington (1935)
Acacia hindsii Benth.	lvs	Seigler et al. (1978)[b]; Rehr et al. (1973); Ebinger & Seigler (1987)
Acacia irrorata Sieber ex Spreng. subsp. *irrorata*	lvs	Conn et al. (1985)
Acacia julifera Benth. subsp. *julifera*	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia kempeana F. Muell.	lvs	Conn et al. (1985); Maslin et al. (1988)

TABLE 1. Continued.

Species	Plant parts	References
Acacia lasiocalyx C. Andrews	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia litakunensis Burch.	lvs, sd	Steyn & Rimington (1935); Steyn (1934b)
Acacia longifolia (Andr.) Willd.[pp]	lvs	Everist (1964); Gardner & Bennetts (1956); Hurst (1942)
Acacia longiphyllodinea Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia lysiphloia F. Muell.	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia macracantha Humb. & Bonpl. ex Willd.	lvs	Seigler et al. (1978)[b]; Seigler & Ebinger (1988)
Acacia milleriana Lundell	lvs	Seigler et al. (1978); Seigler & Ebinger (1988)
Acacia olgana Maconochie	lvs	Conn et al. (1985); Maslin et al. (1988)[ll]
Acacia oswaldii F. Muell.[pp]	lvs	Everist (1964); Hurst (1942)
Acacia pachyphloia	lvs	Conn et al. (1985); Maslin et al. (1985)[b]
Acacia parramattensis Tind.	lvs	Secor et al. (1976); Conn et al. (1985); Maslin et al. (1988)[f]
Acacia pennatula (Schldl. & Cham.) Benth.	lvs	Seigler & Ebinger (1988)[b]
Acacia polybotrya Benth.	lvs	Maslin et al. (1988)[f]
Acacia pringlei Rose	lvs	Seigler et al. (1978)
Acacia pubifolia Pedley	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia pulchella R. Br.	lvs	Secor et al. (1976); Conn et al. (1985)
Acacia pulchella var. *glaberrima*	lvs	Maslin et al. (1988)[c]
Acacia pulchella var. *goadbyi*	lvs	Maslin et al. (1988)[g]
Acacia pulchella ?var. *glaberrima* × var. *goadbyi*	lvs	Maslin et al. (1988)
Acacia pulchella var. *pulchella*	lvs	Maslin et al. (1988)
Acacia pulchella var. *reflexa* Maslin	lvs	Maslin et al. (1988)[mm]
Acacia pulchella var. *subsessilis* Maslin	lvs	Maslin et al. (1988)
Acacia pulchella var. ?	lvs	Maslin et al. (1988)
Acacia pycnostachya F. Muell.	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia resinomarginea W. Fitzg.	lvs	Conn et al. (1985); Maslin & Bennett (1985); Maslin et al. (1988)[g]
Acacia rhodophloia Maslin	lvs	Conn et al. (1985); Maslin et al. (1988)
Acacia robusta Burch.	lvs, frt	Steyn (1934a,b; 1974); Steyn & Rimington (1935)
Acacia roemeriana Scheele	lvs	Seigler (1976b)
Acacia schaffneri (S. Wats.) F.J. Herm. var. *bravoensis* Isely	lvs	Seigler et al. (1978)[b]
Acacia schaffneri var. *schaffneri*	lvs	Seigler et al. (1978)[b]
Acacia cf. *schinoides* Benth.	lvs	Conn et al. (1985); Maslin et al. (1988)[f,ll]
Acacia sibina Maslin	lvs	Conn et al. (1985); Maslin et al. (1988)[ll]

TABLE 1. Continued.

Species	Plant parts	References
Acacia sieberiana DC. var. *woodii* (Burt & Davy) Keay & Brenan[j] (syn.: *Acacia lasiopetala* Oliv.)	lvs, fl, flt, sd	Ettlinger et al. (1977); Nartey et al. (1981); Brimer et al. (1981); Steyn & Rimington (1935); Steyn (1934b); Seigler et al. (1975); Butterfield et al. (1975)
Acacia signata Wendl.	lvs	Conn et al. (1985); Maslin & Bennett (1985); Maslin et al. (1988)[g]
Acacia sparsiflora Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia stowardii Maiden	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia sutherlandii (F. Muell.) F. Muell.	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia tortilis Hayne	lvs, frt, sd, plt	Steyn (1934a)
Acacia tortuosa (L.) Willd.	lvs	Seigler et al. (1983)[b]
Acacia trachycarpa E. Pritzel.	lvs	Conn et al. (1985); Maslin et al. (1988)[oo]
Acacia unijuga Rose	lvs	Ebinger & Seigler (unpublished data)
Acacia yorkrakinensis C. Gardner	lvs	Conn et al. (1985); Maslin et al. (1988)[g]
Acacia aff. *atkinsiana*	lvs	Conn et al. (1985)
Acacia aff. *blakei*	lvs	Conn et al. (1985)[g]
Acacia aff. *citrinoviridis* Tind. & Maslin	lvs	Conn et al. (1985)
Acacia aff. *kempeana*	lvs	Conn et al. (1985)
Acacia aff. *macdonnelliensis* Maconochie	lvs	Conn et al. (1985)
	INGEAE	
Albizia acle Merr.	stbk, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
Albizia procera Benth.		Quisumbing (1949); Herbert (1922)
Albizia saponaria Blume	lvs, bk, rt	Quisumbing (1949); Kalaw & Sacay (1925)
Chloroleucon tortum Pittier (syn.: *Pithecellobium tortum* Mart.)	lvs	Kaplan et al. (1983)
Pithecellobium confine Standl.	lvs	Ebinger & Seigler (unpublished data)
Pithecellobium dulce (Roxb.) Benth.	lvs, bk, rt	Quisumbing (1949); Herbert (1922)
Pithecellobium leiocalyx Standl.	lvs	Ebinger & Seigler (unpublished data)
Pithecellobium saman (Jacq.) Benth. (syn.: *Samanea saman* (Jacq.) Merr. (syn.: *Albizia saman* F. Muell.)	lvs, stbk, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
Wallaceodendron celebicum Koorders	lvs, stbk, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
	MIMOSEAE	
Anadenanthera colubrina (Vell. Conc.) Brenan var. *cebil* (Griseb.) v. Reis (syn.: *A. macrocarpa* (Benth.) Spreng.	lvs	Seigler et al. (1979b)
Dichrostachys cinerea (L.) Wight & Arn.	frt, sd	Gibbs* (1974)
Entada phaseoloides (L.) Merr.	st, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
Leucaena leucocephala (Lam.) deWit (syn.: *L. glauca* (Moench.) Benth.	lvs, st, rt	Quisumbing (1949); Herbert (1922)
Mimosa elliptica Benth.	lvs	Kaplan et al. (1983)
Mimosa pseudo-obovata Taub.	lvs	Kaplan et al. (1983)
Mimosa pudica L.	lvs, st, rt	Quisumbing (1949); Herbert (1922)
Prosopis juliflora (Sw.) DC.	shoot, lvs	Hegnauer (1958)

TABLE 1. Continued.

Species	Plant parts	References
	PARKIEAE	
Parkia javanica Merr.	lvs, bk, rt	Quisumbing (1949)
Parkia timorana Merr.	lvs, bk, rt	Herbert (1922)
	PAPILIONOIDEAE	
	ABREAE	
Abrus precatorius L.	lvs, bk, rt, frt	Quisumbing (1949); Kaplan et al. (1983); Kalaw & Sacay (1925); Herbert (1922)
	ADESMIEAE	
Adesmia bicolor DC.	lvs?	Dominguez et al. (1928)
	AESCHYNOMENEAE	
Arachis hypogaea L.	st, rt	Juliano (1923); Quisumbing (1949)
Stylosanthes viscosa Sw.	lvs	Kaplan et al. (1983)
	BOSSIAEEAE	
Goodia lotifolia Salisb.	lvs	Finnemore & Cox (1928); Finnemore & Large (1936)[k]; Hurst (1942); Everist (1964); Maslin, Conn & Hall (in prep.)
	CICEREAE	
Cicer arietinum	sd	Rosenthaler (1919); Leather (1906)[l]; Montgomery (1964); Wokes & Willimot (1951); Jaffé (1950)
	CORONILLEAE	
Ornithopus compressus L.	sdig, plt	van Valen (1979); Gard (1915)
Ornithopus ebracteatus Brot.	plt	van Valen (1979); Gard (1915)
Ornithopus perpusillus L.	plt	van Valen (1979)[m]; Gard (1915)
Ornithopus pinnatus (P. Mill.) Druce	plt	van Valen (1979)[n]
Ornithopus roseus Dufour.	sdlg, plt	van Valen (1979); Gard (1915)
	CROTALARIEAE	
Crotalaria juncea L.	sd, st	Quisumbing (1949); Juliano (1933)
Lotononis carnosa (Eckl. & Zeyh.) Benth.	plt	Gibbs* (1974)
Lotononis crumaniana	sd, lvs, embryo	Fikenscher & Hegnauer (1981)
Lotononis involucrata (Berg.) Benth.	plt	Steyn (1929)
Lotononis laxa	plt	de Waal (1939)
Lotononis oxyptera (E. Meg.) Benth.	plt	Gibbs* (1974)
	DALBERGIEAE	
Dalbergia ferruginea	lvs, rt	Quisumbing (1949); Juliano (1933)
Geoffroea decorticans (Hook. & Arn.) Burkart	lvs	Seigler et al. (1979b)
Pterocarpus vidalianus	lvs, stbk, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
	DESMODIEAE	
Desmodium gangeticum DC.	lvs, rt	Quisumbing (1949); Juliano (1933)
Desmodium laxiflorum Wight	lvs, st, rt	Quisumbing (1949); Juliano (1933)
Desmodium scorpiurus (SW.) Desv.	lvs, st, rt	Quisumbing (1949); Herbert (1922)
Desmodium triflorum (L.) DC.	lvs	Quisumbing (1949); Juliano (1933)
	GALEGEAE	
Astragalus bergii Hieron.	lvs?	Giusti (1937)
Astragalus canadensis L.	lvs	Seigler (unpublished data)
Astragalus cibarius Sheldon	lvs	Seigler (unpublished data)
Astragalus convallarius Greene	lvs	Seigler (unpublished data)
Astragalus emoryanus (Rydb.) Cory	lvs	Seigler (unpublished data)
Astragalus falcatus Lam.	lvs	Seigler (unpublished data)
Astragalus flexuosus (Hook.) G. Don.	lvs	Seigler (unpublished data)

TABLE 1. Continued.

Species	Plant parts	References
Oxytropis campestris (L.) DC.	lvs	Fikenscher & Hegnauer (1981)
Oxytropis lapponica Gaud.	lvs, sd	Rosenthaler (1919); Greshoff (1909)
Oxytropis sulphurea (Fisch.) Ldb.	lvs, sd, sdlg	Rosenthaler (1919); Gibbs (1974); Greshoff (1909)
	GENISTEEAE	
Lupinus luteus L.	sd	Wokes & Willimot (1951)
Lupinus texensis Hook.	lvs	Seigler (1976b)
	INDIGOFEREAE	
Cyamopsis tetragonolobus (L.) Thunb.	sd	Oke (1964)
Indigofera australis Willd.	lvs	Erroneously attributed to Webb (1949); Hurst (1942); Everist (1964); Fikenscher & Hegnauer (1981)
Indigofera galegoides DC.	lvs	Rosenthaler (1919); van Romburgh (1899); Treub (1907); Quisumbing (1949)
Indigofera suffruticosa P. Mill.	lvs, st, rt, frt	Juliano (1923); Quisumbing (1949)
	LOTEAE	
Dorycnium germanicum (Gremli) Rikli	lvs, st, cotyl.	Plouvier (1974); Fikenscher & Hegnauer (1981)[o]
Dorycnium hirsutum (L.) Ser.	sdlg, but not sd	Fikenscher & Hegnauer (1981)
Dorycnium jordanii Lor. & Bar.	cotyledon sdlg, not sd	Guérin (1929); Plouvier (1974)
Dorycnium pentaphyllum Scop.	sdlg, plant in flower	Fikenscher & Hegnauer (1981)[o]
Dorycnium suffruticosum Vill.	lvs, st, cotyl. sdlg, not sd	Guérin (1929); Plouvier (1974); Fikenscher & Hegnauer (1981)[p]
Lotus alpinus Schleich. & Ramond	lvs	Gibbs (1974); Grant & Sidhu (1967)
Lotus angustissimus L.	sd, plt	Gibbs* (1974)
Lotus arabicus L.	shoot, lvs, sdlg	Gibbs (1974); Grant & Sidhu (1967); Butler (1965); Dunstan & Henry (1900, 1901); Abrol & Conn (1965)[q]; Butler (1965)[q]
Lotus arenarius Brot.	shoot, lvs, sdlg	Gibbs (1974); Grant & Sidhu (1967); Butler (1965)[s]
Lotus australis Andr.	lvs	Maslin, Conn & Hall (in prep.)
Lotus australis var. *pubescens* Benth.	sdlg, lvs	Finnemore & Cooper (1938); Finnemore et al. (1938b)[t]; Webb (1949); Petrie (1912); Everist (1964); Grant & Sidhu (1967)
Lotus biflorus Desv. (syn.: *Tetragonolobus biflorus* Ser.)	sdlg. not sd, cotyledon	Rosenthaler (1929)
Lotus borzii Pitard	lvs	Grant & Sidhu (1967)
Lotus campylocladus Webb & Berth.	shoot, lvs	Grant & Sidhu (1967); Gibbs (1974)
Lotus capillipes Battand. & Trab.		Gibbs* (1974)
Lotus carmeli Boiss.		Gibbs* (1974)
Lotus chihuahuanus Greene	lvs	Grant & Sidhu (1967)
Lotus coccineus Schlecht.	shoot	Gibbs (1974); Everist (1964)
Lotus commutatus Guss.	shoot, lvs	Gibbs (1974); Grant & Sidhu (1967)
Lotus conimbricensis Brot.	sdlg, shoot, lvs	Gibbs (1974); Grant & Sidhu (1967)
Lotus corniculatus L.	plt, not sd, corolla, shoot, lvs	Gibbs (1974); Guérin (1928); Butler (1965)[u]; Petrie (1912); Everist (1964); Grant & Sidhu (1967)

TABLE 1. Continued.

Species	Plant parts	References
Lotus creticus L.	shoot, lvs, sdlg	Gibbs (1974); Guérin (1928); Butler (1965)[v]; Grant & Sidhu (1967)
Lotus cruentus Court.	lvs	Grant & Sidhu (1967); Maslin, Conn & Hall (in prep.)
Lotus cytisoides L.	shoot, lvs	Gibbs (1974); Grant & Sidhu (1967); Guérin (1930)
Lotus drepanocarpus Dur.	shoot	Gibbs (1974)
Lotus edulis L.	shoot, lvs, sdlg	Gibbs (1974); Grant & Sidhu (1967); Butler (1965)[w]; Petrie (1912)
Lotus filicaulis Dur.	shoot, lvs	Gibbs (1974); Grant & Sidhu (1967); Guérin (1930)
Lotus gebelia Vent.	plt, shoot	Guérin (1928); Rosenthaler (1929); Guérin (1929)*
Lotus grandiflorus (Benth.) Greene	lvs	Grant & Sidhu (1967)
Lotus hirsutus L. (syn.: *Dorycnium herbaceum* Vill.) (syn.: *Bonjeania hirsuta* Reich.)	shoot, lvs, st, cotyledon	Rosenthaler (1929); Guérin (1929); Plouvier (1974); Fikenscher & Hegnauer (1981)
Lotus holosericeus Webb & Berth.	lvs	Grant & Sidhu (1967)
Lotus incanus Gray	shoot	Rosenthaler (1919)
Lotus jacobaeus L.	shoot, lvs	Gibbs (1974); Grant & Sidhu (1967); Rosenthaler (1919)
Lotus jolyi Batt.	shoot	Rosenthaler (1929); Foley & Musso (1925)
Lotus krylovii Schischkin & Serg.	plt, lvs	van Valen (1979)[x]; Grant & Sidhu (1967)
Lotus lancerottensis Webb & Berth.	lvs	Grant & Sidhu (1967)
Lotus major Sm.	lvs	Rigg et al. (1933)
Lotus maroccanus Ball	sdlg, shoot, lvs plt, not sd	Butler (1965)[y]; Grant & Sidhu (1967)
Lotus mascaensis Burch.	lvs, shoot	Gibbs (1974); Grant & Sidhu (1967); Guérin (1930)
Lotus mearnsii Britton	shoot	Gibbs (1974)
Lotus nevadensis (S. Wats.) Greene	lvs	Grant & Sidhu (1967)
Lotus ornithopodioides L.	shoot, lvs, plt, sd	Gibbs (1974); Grant & Sidhu (1967); Guérin (1928); van Valen (1979)[z]; Mirande (1913)
Lotus palustris Willd.	lvs	Grant & Sidhu (1967)
Lotus parviflorus Desf.	shoot	Butler (1965)[aa]
Lotus peregrinus L.	young plt, lvs	Gibbs (1974); Grant & Sidhu (1967)
Lotus purshianus (Benth.) Clem. & Clem.	lvs	Grant & Sidhu (1967)
Lotus rectus L. (syn.: *Dorycnium rectum* (L.) Ser.) (syn.: *Bonjeania recta* Reich.)	shoot, cotyledon	Rosenthaler (1929); Guérin (1929)
Lotus requienii Hort. ex. DC.	lvs	Guérin (1928)
Lotus schoelleri Schweinf.	lvs	Grant & Sidhu (1967)
Lotus siliquosus L. (syn.: *Tetragonolobus siliquosus* Roth.)	cotyledon	Rosenthaler (1929); Guérin (1929)
Lotus spartioides Webb. & Berth.	lvs	Grant & Sidhu (1967)
Lotus suaveolens Pers.	lvs	Maslin (unpublished data)
Lotus subpinnatus Lag. (syn.: *Hosackia subpinnata* (Lag.) Torr & Gray)	lvs	Gibbs* (1974)

TABLE 1. Continued.

Species	Plant parts	References
Lotus tenuis Waldst. & Kit. ex Willd.	sdlg, shoot, lvs	Butler (1965)[bb]; Grant & Sidhu (1967); Guérin (1928)
Lotus tetragonolobus L. (syn.: *Tetragonolobus purpureus* Moench.)	lvs, rt	Seigler, unpublished data; van Valen, (1979)[cc]; Guérin, (1929)
Lotus villosus Benth. ex Hook. f	shoot	Rosenthaler (1919); Gibbs (1974)
Lotus weilleri Maire	lvs, shoot	Gibbs (1974); Grant & Sidhu (1967)
Tetragonolobus conjugatus Ser.[ss]	shoot	Gibbs* (1974)
Tetragonolobus requienii (Maur. ex Sang.) Sang.[ss] (syn.: *Tetragonolobus conjugatus* Ser.)	lvs, rt	van Valen (1979)[dd]; Gibbs (1974)
	MILLETTIEAE	
Derris elliptica Benth.	rt	Quisumbing (1949); Juliano (1933)
Derris philippinensis Merr.	lvs, bk, rt	Quisumbing (1949); Herbert (1922)
Mundulea sericea (Willd.) A. Chev.	plt	Gibbs* (1974); Sillans (1952)[qq]
Tephrosia candida (Roxb.) DC.	plt, rt, st, fl	Juliano (1923); Quisumbing (1949)
Tephrosia densiflora Hook. f.	plt	Gibbs* (1974); Sillans (1952)[qq]
Tephrosia macropoda Harvey	lvs	Steyn (1937)
Tephrosia periculosa Baker	lvs	Sillans (1952)[qq]
Tephrosia vogelii Hook. f.	plt	Steyn (1937)
	PHASEOLEAE	
Cajanus cajan	lvs, st, rt	Quisumbing (1949); Montgomery (1964); Jaffé (1950); Herbert (1922)
Canavalia ensiformis (L.) DC.	lvs, rt	Juliano (1923); Quisumbing (1949); Everist (1964); Jaffé (1950)
Canavalia gladiata (Jacq.) DC.	lvs?	Everist (1964)
Canavalia papuana Merr. & Perry	lvs	Maslin, Conn & Hall (in prep.)
Canavalia rosea (Sw.) DC. (syn.: *C. obtusifolia*)	sd, lvs	Kaplan et al. (1983); Freise (1938)
Centrosema plumieri (Turp. ex Pers.) Benth.	plt	Quisumbing (1949); Herbert (1922)
Clitoria sp.	lvs	Kaplan et al. (1983)
Dolichos biflorus L.[rr]	sd	Gibbs* (1974)
Erythrina americana Miller	sd	Lucas & Sotelo (1984)
Erythrina indica Lam.	lvs, st, rt, frt	Herbert (1922)
Erythrina subumbrans (Hassk.) Merr.	lvs, st, rt, frt	Gibbs* (1974)
Erythrina variegata L.	lvs, st, rt, frt	Quisumbing (1949); Kalaw & Sacay (1925)
Erythrina vespertilio Benth.	lvs	Maslin, Conn & Hall (in prep.)
Glycine max	sd	Gibbs* (1974); Honig et al. (1983)
Lablab purpureus (syn.: *Dolichos lablab* L.)	sd, lvs, st, rt	Quisumbing (1949); Leather (1906); Herbert (1922); Rosenthaler (1919); Pammel (1911); Jaffé (1950)
Mucuna pruriens (L.) DC.	frt	Quisumbing (1949); Juliano (1933)
Pachyrrhizus erosus (L.) Urb.	lvs, bk, rt	Quisumbing (1949); Herbert (1922)
Phaseolus coccineus L. (syn.: *P. multiflorus* Willd.)	sd	Wokes & Willimot (1951)*
Phaseolus hintonii	lvs, sd	Delgado (unpublished data)

TABLE 1. Continued.

Species	Plant parts	References
Phaseolus lunatus	lvs, sd	Herbert (1922); Dunstan & Henry (1903) (1904)[ee]; Guignard (1906, 1907); van Romburgh (1899); Treub (1904); Viehoever (1940); Butler (1965)[ee]; Montgomery (1964); Fikenscher & Hegnauer (1981)
Phaseolus lunatus var. *viridis*	lvs, sd	Delgado (unpublished data)
Phaseolus maculatus	lvs, sd	Delgado (unpublished data)
Phaseolus marechalii	lvs, sd	Delgado (unpublished data)
Phaseolus micranthus Hook. & Arn.	lvs, sd	Delgado (unpublished data)
Phaseolus neglectus Hermann	lvs, sd	Delgado (unpublished data)
Phaseolus pauciflorus Benth.	lvs, sd	Delgado (unpublished data)
Phaseolus pedicellatus Benth.	lvs, sd	Delgado (unpublished data)
Phaseolus polystachyus (L.) B.S.P.	lvs, sd	Delgado (unpublished data)
Phaseolus ritensis M.E. Jones	lvs, sd	Delgado (unpublished data)
Phaseolus vulgaris	lvs, sd	Wokes & Willimot (1951); Montgomery (1964, 1965); Jaffé (1950)
Psophocarpus tetragonolobus DC.	lvs, st, rt	Quisumbing (1949); Herbert (1922); Teik (1951)
Teramnus labialis (L.f.) Spreng.	lvs, rt	Quisumbing (1949); Juliano (1933)
Vigna mungo (L.) Hepper (syn.: *Phaseolus mungo* L.)	sd, lvs, fr, rt	Rosenthaler (1919); Leather (1906)[l]
Vigna radiata (L.) R. Wilczek (syn.: *Phaseolus areus* Roxb.) (syn.: *Phaseolus radiatus* Roxb.)		
Vigna unguiculata (L.) Walp. subsp. *unguiculata* (syn.: *V. sinensis* L.)	sd	Montgomery (1964); Jaffé (1950)
ROBINIEAE		
Gliricidia sepium (Jacq.) Steud.	lvs, bk, rt	Quisumbing (1949); Herbert (1922); Lucas & Sotelo (1984)
Sesbania grandiflora (L.) Pers.	frt, stbk, rtbk, plt	Quisumbing (1949); Juliano & Guerrero (1935)
Sesbania punicea (Cav.) Benth.	sd	da Costa Sacco (1957); Giusti (1938)
SOPHOREAE		
Holocalyx balansae Mich.	lvs, sd	Seigler et al. (1979b); Nahrstedt (1976)[ff]; Gmelin et al. (1973)[gg]
Holocalyx glaziovii Taub.		Burkart (1952); Machado & Occhioni (1943)
TRIFOLIEAE		
Medicago sativa L.	lvs	Hegnauer (1959)[hh]; Rosenthaler (1923); Rigg et al. (1933); Steyn (1937); Harris et al. (1980)
Melilotus officinalis (L.) Pallas	plant in bloom	Rosenthaler (1923)
Trifolium ambiguum Bieb.	lvs	Gibson et al. (1972)
Trifolium hybridum L.	lvs	Rigg et al. (1933)
Trifolium isthmocarpum Brot.	lvs	Gibson et al. (1972); Hegnauer (1961)
Trifolium lupinaster L.	lvs, bd, plt	Egorov et al. (1965)
Trifolium meneghinanum Clem.	lvs	Gibson et al. (1972)
Trifolium montanum L.	lvs	Gibson et al. (1972)
Trifolium nigrescens Vis.	plt	van Valen (1979)[ii]; Gibson et al. (1972)

TABLE 1. Continued.

Species	Plant parts	References
Trifolium occidentale Coombe	lvs	Gibson et al. (1972)
Trifolium petrisavii Clem.	lvs	Gibson et al. (1972)
Trifolium pratense L.	lvs, buds	Rosenthaler (1923); Rigg et al. (1933)
Trifolium repens L.	plt, flower	Melville & Doak (1940)[ii]; Tapper & Reay (1973); Finnemore et al. (1938a); Everist (1964); Butler (1965)[kk]
Trifolium subterraneum L.	lvs	Rigg et al. (1933)
Trifolium uniflorum L.	lvs	Gibson et al. (1972)
Trigonella suavissima Lindl.	lvs	Maslin (unpublished data)
	VICIEAE	
Lens culinaris Medik.	sd	Jaffé (1950)
Pisum sativum L.	lvs, st, rt, frt, sd	Montgomery (1965); Wokes & Willimot (1951); Jaffé (1950)
Vicia ambigua Guss.	sd	Rosenthaler (1928, 1929)
Vicia amoena Fisch. ex Ser.	lvs, bd, plant in flower	Egorov et al. (1965)
Vicia benghalensis L. (syn.: *V. atropurpurea* Steud.)	sd	Rosenthaler (1928, 1929)
Vicia biennis L.	sd	Rosenthaler (1928, 1929)
Vicia canadensis Zucc.	sd	Bruyning & van Haarst (1899)
Vicia cassubica L.	sd	Rosenthaler (1928, 1929)
Vicia cornigera Chaub.	sd	Rosenthaler (1928)
Vicia cracca L.	plant in bloom	Rosenthaler (1923)
Vicia cracca subsp. *gerardii* (syn.: *V. gerardii* Vill.)	sd	Rosenthaler (1928, 1929)
Vicia disperma DC.	sd	Rosenthaler (1928, 1929)
Vicia ervilia (L.) Willd.	cotyledons	Gibbs* (1974)
Vicia faba L.	sd	Wokes & Willimot (1951); Cugudda et al. (1953)
Vicia globosa Retz.	sd	Rosenthaler (1928, 1929)
Vicia grandiflora Scop.	sd	Tschiersch & Hanelt (1967); Rosenthaler (1928)
Vicia hirsuta (L.) S.F. Gray	sd	Rosenthaler (1919, 1928); Everist (1964); Gibbs (1974); Bruyning & van Haarst (1899)
Vicia lutea L.	sd	Rosenthaler (1928, 1929)
Vicia michauxii Schrank.	sd	Rosenthaler (1928, 1929)
Vicia monantha Retz. (syn.: *V. calcarata* Desf.)	sd	Rosenthaler (1928, 1929); Everist (1964)
Vicia multiflora Lam.	sd	Rosenthaler (1928, 1929)
Vicia narbonensis L.	sdlg	Rosenthaler (1928, 1929)
Vicia pannonica Crantz. (syn.: *Vicia striata* Bieb.)	sd	Rosenthaler (1928, 1929)
Vicia peregrina L.	sd	Tschiersch & Hanelt (1967); Bruyning & van Haarst (1899); Ritthausen & Kreusler (1870); Montgomery (1965, 1964); Everist (1964); Wokes & Willimot (1951)
Vicia sativa	sd, cotyledon	Tschiersch & Hanelt (1967)
Vicia sativa subsp. *cordata* (syn.: *V. cordata* Wulf.)	sd	

TABLE 1. Continued.

Species	Plant parts	References
Vicia sativa subsp. *macrocarpa* (syn.: *V. macrocarpa* (Moris) Bert.	sd, cotyledon	Bertrand & Rivkind (1906); Guignard (1906); Tschiersch & Hanelt (1967)
Vicia sativa subsp. *nigra* (syn.: *V. angustifolia* L.)	sd	Bertrand (1906); Chaudhury & Robertson (1949); Everist (1964); Tschiersch & Hanelt (1967); Bruyning & van Haarst (1899)
Vicia sepium L.	sd	Rosenthaler (1923, 1928, 1929)
Vicia tricolor Schur.	sd	Rosenthaler (1928, 1929)
Vicia villosa Roth.	sd from blackened pod	Rosenthaler (1919, 1928); Armstrong et al. (1912)
Vicia villosa subsp. *pseudocracca* (syn.: *V. pseudocracca* Bertol.)	sd	Rosenthaler (1928, 1929)
Vicia villosa subsp. *varia* (syn.: *V. polyphylla* Waldst. et. Kit.)	sd	Rosenthaler (1928, 1929)

bk = bark, cotyl = cotyledon, fl = flower, frt = fruit, lvs = leaves (or phyllodes), plt = plant, rt = root, rtbk = rootbark, sd = seed, sdlg = seedling, st = stem, stbk = stem bark.

*Attributed to "others" by Gibbs (1974).

[a]This species contains a mixture of linamarin and lotaustralin.

[b]This species contains proacacipetalin.

[c]This taxon contains sambunigrin.

[d]Material from the plant used in this study was identified as *Acacia blakei* by L. Pedley of the Queensland Herbarium.

[e]This name has been applied in an extremely broad sense in the past. It is not now possible to determine with certainty the identity of plants described as toxic with this name (cf. Conn et al., 1985).

[f]This species contains prunasin.

[g]This taxon contains predominately sambunigrin with a lesser amount of prunasin.

[h]This species contains epiproacacipetalin.

[i]Extensive tests indicate that this species is rarely, if ever, cyanogenic. In the original reference, the species is only identified as "catclaw" acacia. Another species in the same area described, *Acacia constricta*, is usually cyanogenic.

[j]This species contains proacacipetalin, 3-hydroxyheterodendrin, proacaciberin, and heterodendrin.

[k]This species contains a compound that was characterized as *p*-glucosyloxymandelonitrile.

[l]Although several have attributed these to Leather (1906), there is no mention of them in his article.

[m]This plant contains a mixture of linamarin (69%) and lotaustralin (31%).

[n]This plant contains a mixture of linamarin (97%) and lotaustralin (3%).

[o]This species contains nearly linamarin free lotaustralin.

[p]Lotaustralin was isolated from this species.

[q]This plant contains linamarin (70%) and lotaustralin (30%).

[r]This species contains a 95:5 mixture of lotaustralin and linamarin.

[s]This species contains linamarin (99%) and lotaustralin (1%).

[t]This plant primarily contains lotaustralin.

[u]This species contains linamarin (11–57%) and lotaustralin (43–89%).

[v]This species contains linamarin (84%) and lotaustralin (16%).

[w]This species contains linamarin (79%) and lotaustralin (21%).

[x]This plant contains a mixture of linamarin (8%) and lotaustralin (92%).

[y]This species contains linamarin (58–74%) and lotaustralin (26–42%).

[z]The leaves contain linamarin (96%) and lotaustralin (4%) and the seeds linamarin (98%) and lotaustralin (2%).

[aa]This species contains linamarin (21%) and lotaustralin (79%).

[bb]This species contains linamarin (6%) and lotaustralin (94%).
[cc]This plant contains a mixture of linamarin (75%) and lotaustralin (25%).
[dd]The leaves contain linamarin (88%) and lotaustralin (12%) and the roots linamarin (80%) and lotaustralin (20%).
[ee]This species contains linamarin (92–96%) and lotaustralin (4–8%).
[ff]The leaves of this species contain prunasin.
[gg]The seeds of this species contain holocalin.
[hh]This plant is cyanogenic only in wilted condition.
[ii]This plant contains a mixture of linamarin (6%) and lotaustralin (94%).
[jj]This plant contains mixtures of linamarin (20–25%) and lotaustralin (75–80%).
[kk]This species contains linamarin (26–50%) and lotaustralin (50–74%).
[ll]This species contains prunasin with a lesser amount of sambunigrin.
[mm]This taxon contains heterodendrin.
[nn]Contains small amounts of an apparently aliphatic cyanogen.
[oo]Equal or almost equal amounts of sambunigrin and prunasin.
[pp]Recent tests suggest that cyanogenesis is not found in this species (Conn et al., 1985).
[qq]Mentioned in Sillans (1952), but the citation is ambiguous.
[rr]*Dolichos biflorus* of this report may be *Vigna unguiculata* or *Macrotyloma uniflorum* (Lam.) Verdc.
[ss]These species will ultimately have to be transferred to the genus *Lotus*.

In most instances, cyanogenic compounds probably are lacking, but many other factors may be involved. We have not included negative reports in this work.

Sometimes negative tests result from improper preparation of samples. Some cyanogenic compounds and most of the hydrolytic enzymes are sensitive to heat and the action of fungi, bacteria, and other agents. For example, air-dried plants of *Lotus australis* lost as much as 60% of their original ability to liberate HCN (Finnemore & Cooper, 1938). The age of specimens alone does not seem to be a major factor, as good-quality herbarium materials over 100 years old frequently are cyanogenic without addition of exogenous enzyme.

Variation in Cyanogenesis in Plants

Although almost all individuals of some species are strongly cyanogenic and most species and genera of some families are cyanogenic, variation and polymorphism in the presence/absence and amounts of cyanogenic compounds and their hydrolytic enzymes is a common situation (e.g., Saupe, 1981; Fikenscher & Hegnauer, 1981; van Valen, 1979). Failure to obtain positive tests (even of species well known to be cyanogenic) may be attributable to many factors.

Plants of *Acacia farnesiana* (L.) Willd. from Costa Rica varied considerably in the content of cyanogenic compounds. However, plants that contained large amounts of these compounds in one season tended to contain high amounts in the next season, although the absolute amounts present differed from season to season (Seigler et al., 1979a; Janzen et al., 1980).

In a survey of a large number of Australian species of *Acacia*, much variation in the presence/absence of cyanogenesis has been reported (Conn et al., 1985). Few Australian species of *Acacia* possess the β-glucosidase necessary to hydrolyze the cyanogenic glycosides present (Finnemore & Gledhill, 1928a, b; Conn & Maslin, 1982; Conn et al., 1985), and exogenous enzyme must be added to test for the presence of HCN from these species. In populations of *A. gracillima* Tind., *A. resinomarginea* W. Fitzg., and *A. trachycarpa* E. Pritzel, the percentage of cyanogenic individuals varied from 0% to 35%. In populations of *A. signata* F. Muell., however, all plants tested were positive. Maslin & Bennett (1985) showed that plants of *Acacia signata* (20 of 20 plants tested) and *A. resinomarginea* (9 of 20 tested) gave the strongest positive reactions in spring–summer corresponding with the period of flowering, fruiting, and new shoot production. Weaker reactions were recorded for autumn–winter when the plants were dormant. However, the production of cyanogenic glycosides in these *Acacia* species seems to be independent of age.

Cyanogenic polymorphism detectable in *Trifolium repens* L. is controlled by two independent loci. One allelic pair controls production of the glucosides linamarin and lotaustralin, and the other governs β-glucosidase activity (Corkill, 1942; Gibson et al., 1972; Jones, 1981). Plants with the glycoside and enzyme, glycoside only, enzyme only and neither are known to occur. The genetic control of cyanogenesis and the ecological role of this variation have been reviewed (Hughes, 1981; Jones, 1981). Genetic studies of most other cyanogenic plants have not been conducted and the importance and nature of genetic control of cyanogenesis is not known.

Other changes in cyanogenic activity are associated with stage of development of the plants concerned. The percentage of HCN liberated from very young leaves of *Phaseolus lunatus* L. (0.32%) decreased as the leaves matured (0.009%) and were about to fall. In contrast, the change in leaves of *Indigofera galegoides* was much smaller (0.15% to 0.11%) (Treub, 1907). The presence of the cyanogens of *Lotus australis* Andr. is restricted to actively growing parts of the plant, and these compounds are not found in the seeds (Dunstan & Henry, 1903). Populations of seedlings from two locations in Western Australia contained about 15% acyanogenic individuals. However, only 2% of the total plants examined failed to develop the glycosides and enzymes by time of flowering (Foulds, 1982). The amount of cyanide produced by plants of *Phaseolus lunatus* varies during the day. A general increase at midday and at 6 p.m. compared with the morning has been reported (Treub, 1904). This paper might not hold up to modern statistical treatment.

The Value of Cyanogenesis for Systematic Studies

The value of cyanogenesis in systematics depends on the type of study conducted. The information content is greater for structural information on cyanogenic compounds than for presence/absence data for hydrogen cyanide. Studies involving the structures of compounds involved, precursors, sequence of intermediates involved in the formation, and the nature of the enzymes and enzyme complexes involved are of even more value (Saupe, 1981; Hegnauer, 1977).

Simple presence/absence data have proven useful in a number of studies. Genera such as *Passiflora* (Passifloraceae) and *Prunus* (Rosaceae) are virtually always cyanogenic. Individual specimens of *Acacia constricta* are always positive for cyanogenic compounds, whereas the closely related species *Acacia neovernicosa* is consistently negative.

About 55 cyanogenic compounds are known from higher plants (Seigler, 1981). In instances where the parent cyanogenic compounds have been identified, there is little question that the plant is or at least has the potential of being cyanogenic. This character provides a more useful source of taxonomic information than does the presence of cyanogenesis alone. Variations in the stereochemistry, aglycone portions, and glycosidic portions of the isolated cyanogenic compounds are useful in establishing relationships among groups of cyanogenic plants.

Species of *Acacia* belonging to the primarily Australian subg. *Phyllodineae* sections *Pulchellae*, *Botrycephalae*, and *Phyllodineae* contain the cyanogenic glycosides sambunigrin and prunasin, whereas those of subg. *Acacia* (American, African, and Asian for the most part) contain proacacipetalin, epiproacacipetalin, linamarin, lotaustralin, and similar compounds.

Structural differences are useful and confirm that the *Acacia* species belong to two different groups; studies of the biosynthesis of the cyanogens of the two groups have identified the precursors and provided other related information about the formation of the compounds. These studies are more significant and provide much more information than structures alone.

Study of the pathways leading to the cyanogens provides even more information since structures of precursor amino acids and intermediates in addition to the final product may be useful. Relationships between cyanogenic compounds and other groups of secondary compounds (such as nonprotein amino acids, nitro glycosides, and others) may be seen that would otherwise be obscured.

Cyanogenic Glycosides from the Leguminosae

With the exception of proacacipetalin, epiproacacipetalin, proacaciberin, and 3-hydroxyheterodendrin from members of the genus *Acacia,* no unique cyanogenic compounds are known from Leguminosae. Prunasin has been reported from the Passifloraceae, Rosaceae, Euphorbiaceae, Compositae, Myrtaceae, and other families; sambunigrin from the Caprifoliaceae, Olacaceae, Rutaceae; vicianin from several species of ferns; holocalin from the Caprifoliaceae; *p*-glucosyloxymandelonitrile from the Berberidaceae; and linamarin and lotaustralin from the Passifloraceae, Linaceae, Euphorbiaceae, and Compositae (Saupe, 1981; Seigler, 1981). The utility of these structural data are limited, however, because of the small number of compounds that have been isolated and characterized from legumes.

The known cyanogenic compounds in the Leguminosae are presumed to arise from the amino acids phenylalanine, tyrosine, leucine, valine, and isoleucine (Conn, 1981; Saupe, 1981). Compounds that appear to be derived from valine and isoleucine probably represent only one biosynthetic pathway as the compounds usually co-occur, and only one set of enzymes appears to be involved in their formation (Conn, 1981).

Based on a critical evaluation of the literature, the current state of knowledge of various aspects of cyanogenesis in the subfamilies and tribes of the Leguminosae is summarized below. In Table 1, all known reports of cyanogenesis in species of the Leguminosae are listed. As discussed above, a number of these reports are questionable.

Caesalpinioideae

Amherstieae:

Only one, dubious, report is known. No compounds have been identified.

Caesalpinieae:

Only three reports are known and are dubious. No compounds have been identified.

Cassieae:

Although most reports from the group are dubious, records from *Cassia* sens. lat. species suggest cyanogenesis but should be confirmed. No compounds have been identified.

Cercideae:

Most records from this tribe are dubious. The report of cyanogenesis from *Bauhinia carronii* F. Muell. warrants reexamination. No compounds have been identified.

Detarieae:

The only report from this tribe is dubious. No compounds have been identified.

Mimosoideae

Acacieae:

Many species of *Acacia* (the only genus in this tribe) are cyanogenic. At least nine cyanogenic compounds have been reported from members of this widely distributed genus. Sambunigrin and prunasin (derived from the aromatic amino acid phenylalanine) are normally the only cyanogens found in Australian members of subg. *Phyllodineae* sections *Pulchellae, Botrycephalae,* and *Phyllodineae.* A series of compounds derived from aliphatic amino acids (linamarin, lotaustralin, proacacipetalin, epiproacacipetalin, heterodendrin, proacaciberin, and 3-hydroxyheterodendrin) are

found in members of subg. *Acacia* from the New World and the Old World. Recently one Australian species of this subgenus, *Acacia pachyphloia* W. Fitzg. ex Maiden, was also found to contain proacacipetalin (Maslin et al., 1985). Even though several species of subg. *Aculeiferum* have been reported to be cyanogenic, no successful isolation and structure determination has been reported. No reports of cyanogenesis from plants formerly considered to belong to the *Filicinae* are known.

The distribution of the major pathways leading to the production of cyanogenic compounds (i.e., those derived from aromatic amino acids as opposed to those derived from aliphatic amino acids) generally correlates with currently accepted taxonomic concepts (Seigler & Conn, 1982). Recently, however, heterodendrin, an aliphatic cyanogenic compound, has been found in *Acacia exilis* Maslin and in *A. pulchella* R. Br. var. *reflexa* Maslin, although *A. pulchella* var. *glaberrima* Meisn. and var. *goadbyi* (Domin.) Maslin contain only the aromatic cyanogen sambunigrin.

Ingeae:

Most reports of cyanogenesis from this tribe must be considered questionable as the method used for determination of cyanogenesis is of doubtful validity. Two reports from *Pithecellobium*, however, suggest that cyanogenesis does occur in the Ingeae. The compounds responsible have not been identified.

Mimoseae:

Reports from *Anadenanthera, Dichrostachys, Mimosa,* and *Prosopis* confirm the presence of cyanogenic compounds within the group. No compounds have been identified.

Parkieae:

Only two dubious reports are known. No compounds have been identified.

PAPILIONOIDEAE

Abreae:

Most reported tests are dubious. One recent report suggests that the group should be reexamined. No compounds have been identified.

Adesmieae:

There is only one dubious report from this tribe. No compounds have been identified.

Aeschynomeneae:

Most reported tests are dubious. Again, a recent report from *Stylosanthes* suggests that the group should be investigated more closely. No compounds have been reported.

Bossiaeeae:

Leaves, twigs, and green fruits of *Goodia lotifolia* Salisb. have been shown to contain large quantities of an unusual labile glycoside that largely disappears upon drying. This compound is *p*-glucosyloxymandelonitrile (Finnemore & Large, 1936; Hurst, 1942). As some residual activity remains in dried material, it is probable that other cyanogenic compounds are also present. The cyanogens found in this tribe appear to be tyrosine derived.

Cicereae:

The seeds of *Cicer arietinum* L. are weakly cyanogenic. No compounds have been identified.

Coronilleae:

All known cyanogenic species of this tribe belong to the genus *Ornithopus*. Leaf material of several species contains the active compounds, but seeds do not (van Valen, 1979). Some plants lack the hydrolytic enzyme necessary to cleave the compounds; added linamarase released cyanide

in some instances (van Valen, 1979). Organic nitro compounds have been reported to occur in *Coronilla varia* L. (not known to be cyanogenic) (Moyer et al., 1977).

Crotalarieae:

Several species of the East African and South African genus *Lotononis* are strongly cyanogenic. The glycoside from *L. laxa* Eckl. & Zeyh. (not isolated) produced hydrogen cyanide and benzaldehyde as cleavage products (de Waal, 1939). The embryos, seeds, and leaves of *L. crumaniana* Burch. ex Benth. also yielded hydrogen cyanide and benzaldehyde (identified as the 2,4-dinitrophenylhydrazone) (Fikenscher & Hegnauer, 1981). The rapidity of hydrolysis with emulsin led these workers to conclude that the cyanogen was prunasin. In any case, the cyanogens of this tribe that have been examined appear to be derived from phenylalanine.

Dalbergieae:

Most tests within this tribe are dubious. The Argentine species *Geoffroea decorticans* Gill. ex (Hook. & Arn.) Burkart is weakly cyanogenic. No compounds have been identified.

Desmodieae:

Tests of plants from this tribe are dubious. No compounds have been identified.

Galegeae:

The presence of cyanogenic glycosides in *Oxytropis campestris* (L.) DC. varies in different accessions (Fikenscher & Hegnauer, 1981). The level of cyanide declines as plants approach the blooming stage. A compound isolated from this species is cleaved by emulsin and by linamarase. Hydrolysis of the glycoside and preparation of a 2,4-dinitrophenylhydrazone suggests that the compound is heterodendrin or epiheterodendrin (Fikenscher & Hegnauer, 1981). These compounds are otherwise known only among the legumes from *Acacia*. In another accession, compounds based on aromatic amino acids were observed. The major glycoside appeared to be zierin accompanied by a lesser amount of sambunigrin (Fikenscher & Hegnauer, 1981).

Numerous species of *Astragalus* contain 3-nitro-1-propanoic acid and related compounds (Stermitz, 1977). These compounds are related biosynthetically to cyanogenic glycosides (Hösel et al., 1985) and in some instances give rise to false positive tests for cyanide with sodium picrate paper. We also have demonstrated recently that 3-nitropropionic acid also gives a false positive Feigl Anger test. In order to establish unambiguously the presence of cyanogenesis in *Astragalus*, identification of specific cyanogenic compounds will be necessary.

Genisteae:

Two *Lupinus* species have been reported to be cyanogenic. No compounds have been identified.

Indigofereae:

Fresh plant material of *Indigofera galegioides* DC. contains a glycoside that yields hydrogen cyanide and benzaldehyde upon addition of emulsin (van Romburgh, 1899). This suggests the presence of prunasin or sambunigrin. Material of *I. australis* Willd. studied by Fikenscher & Hegnauer (1981) proved to be only very weakly cyanogenic and possibly give false positive tests with the Feigl Anger method.

Indigofera endecaphylla Jacq. and *I. subulata* Poir. contain β-nitropropionic acid (hiptagenic acid), which is related to karakin and hiptagin (Hegnauer, 1959). Several compounds of this type give false positive tests with the sodium picrate and the Feigl Anger method.

Loteae:

The systematic significance of the presence of cyanogenic glycosides in the Loteae has been reviewed (van Valen, 1979). Cyanogenesis is common and widespread among members of the

genus *Lotus*. Most of the Old World species tested by Grant & Sidhu (1967) contained cyanogenic glycosides, whereas most of the New World species lacked them. Although *Dorycnium, Tetragonolobus, Hosackia,* and *Bonjeania* have been considered congeneric with *Lotus* (Polhill & Raven, 1981), one could make a case for maintaining *Hosackia* and *Tetragonolobus* based on the general absence of cyanogenic compounds in this group of plants (Grant & Sidhu, 1967; Hegnauer, 1971). A number of species referable to the genera *Dorycnium, Tetragonolobus,* and *Bonjeania* have been reported to be cyanogenic. Lotusin, reported by Dunstan & Henry (1901) was later shown to be a mixture of the flavonoids quercetin and kaempferol and lotaustralin (Abrol & Conn, 1965). The relative amounts of linamarin and lotaustralin in members of the Loteae vary, but individuals of most species contain both compounds (Butler, 1965). In the genus *Lotus,* cotyledons of germinating seeds and whole young plants are strongly cyanogenic (Guérin, 1928, 1929). The concentration increases in the leaves until flowering and then declines. Seeds of *L. ornithopodioides* L. are cyanogenic (van Valen, 1979). Two accessions of *Dorycnium hirsutus* L. lost their cyanogenic properties quickly after the embryonic stage, although two others remained strongly cyanogenic if linamarase was added. The cyanogens of *D. rectum* (L.) Ser. (= *Lotus rectus* L.) were only short-lived. In *D. pentaphyllum* Scop., however, even blooming plants were still strongly cyanogenic. Members of the *Anthyllis, Securigera,* and *Hymenocarpos* are never cyanogenic (Guérin, 1928, 1929; Hegnauer, 1959). The only pathway that appears to be found in the Loteae is based on valine and isoleucine (Fikenscher & Hegnauer, 1981).

Millettieae (Tephrosieae):

Most reports of cyanogenesis from this tribe are dubious. No compounds have been identified.

Phaseoleae:

Linamarin and smaller amounts of lotaustralin (4–8%) were found in samples of *Phaseolus lunatus* investigated by Butler (1965). Several other species of this group have economic importance. Although the presence of cyanogenic materials has been reported from *Cajanus cajan* (L.) Hath, *Glycine max* (L.) Merr., *Lablab purpureus* (L.) Sweet, *Phaseolus coccineus* L., *Phaseolus vulgaris* L., *Vigna mungo* (L.) Hepper, *Vigna radiata* (L.) Wilczek, and *Vigna unguiculata* (L.) Walp., the amounts appear to be very small and no cyanogenic compounds have been isolated. Several of the reports are dubious and should be confirmed in any case.

The wound sap of *Phaseolus lunatus* has been reported to contain such fungitoxic materials as vanillin, *p*-hydroxybenzaldehyde, and their strongly active cyanohydrins (Bell, 1967, 1970). The leaves contain two glycosides that give rise to hydrogen cyanide, sugar, and *p*-hydroxybenzaldehyde. These compounds are presumably derived from tyrosine and serve as precursors of the two cyanohydrins. Vanillin cyanohydrin may arise from reaction of the free aldehyde and cyanide. Fikenscher & Hegnauer (1981) isolated only linamarin. They observed rapid hydrolysis with linamarase but no reaction with emulsin.

Leaves of an *Erythrina* species from Java produce N_2O_3 and acetone (Hegnauer, 1959).

Robinieae:

Most reports of cyanogenesis in this group are doubtful. A report from the seeds of a Brazilian *Sesbania* species suggests the group should be reexamined. No compounds have been identified.

Sophoreae:

The leaves of *Holocalyx balansae* Mich. contain prunasin, and the seeds contain holocalin. Prunasin is derived from phenylalanine, and, although it has not been demonstrated conclusively, the occurrence of holocalin and prunasin in the same species suggests strongly that holocalin also comes from prunasin. A cyanogenic glycoside, holocalin is rare in nature and has only been reported otherwise from the Caprifoliaceae. It is an epimer of zierin, known from the Rutaceae.

Trifolieae:

Several species of this tribe are known to be cyanogenic; most of them belong to *Trifolium*. The compounds responsible in most if not all of these are linamarin and lotaustralin. As is true in other groups, these compounds co-occur in varying ratios (Butler, 1965). In *Trifolium repens* (white clover) polymorphism is governed by two independent loci. One allelic pair controls production of linamarin and lotaustralin and another the presence of β-glucosidase. Cyanogenesis only results when the cyanogens and the enzyme are both present (Corkill, 1942; Gibson et al., 1972). These genes are expressed in seedlings and in mature plants.

Vicieae:

Seeds of many species of *Vicia* contain the aromatic cyanogenic compound vicianin. Species of *Vicia* that contain cyanogenic glycosides belong to the same subgroup characterized by the occurrence of the nonprotein amino acid β-cyanoalanine. These species are all closely related to *Vicia sativa* L. (Hanelt & Tschiersch, 1967). Although many members of the genus *Lathyrus* contain nonprotein amino acids and the genus is often considered to be related to *Vicia*, no members of the genus *Lathyrus* have been reported to be cyanogenic.

Conclusions

The Caesalpinioideae and Mimosoideae

The ability to release cyanide has been reported from all five tribes of the Caesalpinioideae: the Amherstieae, Caesalpinieae, Cassieae, Cercideae, and Detarieae. However, the property seems to be sporadically distributed, and many of the reports are dubious. Cyanogenic compounds have not been isolated from this subfamily.

Cyanogenic compounds have been reported from four of the five tribes of the Mimosoideae: the Acacieae, Ingeae, Mimoseae, and Parkieae. The Mimozygantheae (with the one genus *Mimozyganthus*) apparently have not been examined carefully, although leaves from herbarium material do not appear to be cyanogenic. *Acacia* has been studied extensively. No cyanogenic compound from the tribes other than Acacieae has been isolated.

Data for Mimosoideae and Caesalpinioideae reveal little concerning the systematic significance of cyanogenic compounds in these subfamilies, with the exception of *Acacia* (Seigler & Conn, 1982; Maslin et al., 1988). Many of the dubious records must be reconfirmed, and the compounds responsible must be isolated and characterized.

The Papilionoideae

Our knowledge of cyanogenesis in the Papilionoideae is more informative than that of the Caesalpinioideae and the Mimosoideae, but even in this group, many dubious records should be reconfirmed, and compounds should be isolated and characterized. A number of tribes need to be surveyed more extensively.

In the Papilionoideae, cyanogenic compounds have been reported from 19 of the 31 tribes. The ability to produce HCN upon hydrolysis has been reported from the Abreae, Adesmieae, Aeschynomeneae, Bossiaeeae, Cicereae, Coronilleae, Crotalarieae, Dalbergieae, Desmodieae, Galegeae, Genisteae, Indigofereae, Loteae, Millettieae Phaseoleae, Robinieae, Sophoreae, Trifolieae, and Vicieae but not from the Amorpheae, Brongniartieae, Carmichaelieae, Dipterygeae, Euchresteae, Hedysareae, Liparieae, Mirbelieae, Podalyrieae, Psoraleeae, Swartzieae, and Thermopsideae. In some instances these reports are questionable and should be reexamined. If one eliminates the reports of Herbert (1922), de Peralta (1928), Quisumbing (1949), Juliano (1923, 1933), Juliano &

Guerrero (1935), Kalaw & Sacay (1925), the tribes Parkieae (Mimosoideae), Amherstieae, Caesalpinieae, Detarieae (Caesalpinioideae), and Desmodieae (Papilionoideae) are without positive reports. In several other tribes, reports by this group of workers constitute most of the taxa reported positive.

It has been suggested that a close relationship exists between the metabolism of cyanogenic compounds and organic nitro and related compounds (Hösel et al., 1985; Hegnauer, 1959). As some of these compounds give false positive HCN tests with the Feigl Anger and with the picrate method, reports from *Indigofera* and *Astragalus* species (as well as some species of the Loteae and Coronilleae) should be reconfirmed. Identification of cyanogenic glycosides is especially important in these groups to establish the presence of cyanogenesis.

Most of the tribes that commonly have cyanogenic members are considered to be evolutionarily advanced (Polhill, 1981). The Coronilleae, Loteae, and Trifolieae (all of which contain linamarin and/or lotaustralin) are generally considered closely related. They are placed proximally to the Cicereae and Vicieae in the diagram of tribal relationships (Polhill, 1981, p. 199). Although the cyanogenic compounds of *Cicer* have not been studied, vicianin is commonly found in *Vicia* of the Vicieae. It is probable that few representatives of the Hedysarieae have been tested. Although several representatives of the Galegeae are known to be cyanogenic, the compounds are not known with certainty. One accession of *Oxytropis campestris* appeared to have compounds resembling heterodendrin or epiheterodendrin, whereas another appeared to have a compound resembling zierin or holocalin (Fikenscher & Hegnauer, 1981). Members of this key group should be reinvestigated.

At least one member of the Crotalarieae appears to contain prunasin. This tribe also appears to be relatively advanced compared with Liparieae and Podalyrieae, which apparently lack cyanogenic compounds.

Members of the Indigofereae appear to contain prunasin or sambunigrin (Fikenscher & Hegnauer, 1981). A number of species of the Phaseoleae are cyanogenic. Linamarin and lotaustralin have been identified from this tribe. The Psoraleae and Amorpheae appear to lack cyanogenic compounds, and reports from the Desmodieae should be reinvestigated. All of these tribes are derived from a basal group, the Millettieae (Tephrosieae), from which only dubious HCN positive records are known (no compounds identified). Members of the Robinieae and Aeschynomeneae (also putatively derived from the Millettieae) have been reported cyanogenic, but no compounds from these tribes have been characterized.

One member of the Australian tribe Bossiaeeae contains a most unusual cyanohydrin and is alone in that characteristic in the Leguminosae. *Holocalyx balansae*, a member of the Sophoreae (represented as relatively primitive in Polhill's diagram) contains prunasin and holocalin.

EVOLUTIONARY SIGNIFICANCE OF CYANOGENESIS

Although the pathways leading to production of cyanogenic compounds in the Papilionoideae are relatively short and it could be argued that they are simple processes that may have evolved many times, the biosynthetic enzymes, organization, and types of chemical intermediates of pathways that have been studied are extremely similar if not identical. Is it highly probable that these pathways had a common origin.

Many species of the Rosaceae and Sapindaceae are cyanogenic and possess structurally similar compounds that are derived from virtually identical pathways. As these two families are often considered to be related to the Leguminosae, it is probable that the common ancestors of all three families possessed these pathways. Most of the tribes considered "primitive" in the Papilionoideae (see p. 199 in Polhill & Raven, 1981) do not accumulate cyanogenic glycosides. Thus, it appears that the ability to synthesize or accumulate cyanogenic glycosides has been lost or suppressed. The mechanisms involved in this phenomenon are unknown. Most of the cyanogenic species belong to tribes that are considered evolutionarily advanced. Most of these are found in the temperate zones

of the northern and southern hemispheres. The ability to produce and accumulate cyanogenic glycosides in these divergent lines appears to be "unrepressed." The many acyanogenic representatives in these advanced temperate lines probably represent an additional level of repression.

Most of the groups in which cyanogenesis is common are temperate plants. Although there is undoubtedly a bias toward testing of temperate plants, cyanogenesis does not appear to be as common in tropical representatives of the family. Perhaps the ability to accumulate cyanogenic compounds is a response to colder climates and modified ecological conditions (Jones, 1981) than those faced by the more tropical ancestors of the Leguminosae.

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References

Abrol, Y. P. & E. E. Conn. 1965. Non-occurrence of lotusin in *Lotus arabicus*. Nature 206: 399.

Armstrong, H. E., E. F. Armstrong & E. Horton. 1912. Studies on enzyme action. XVII. Enzymes of the emulsin type. (II) The distribution of β-enzymes in plants. Proc. Roy. Soc. Lond. 85B: 363–369.

Bell, A. A. 1967. Formation of fungitoxins in wound sap of *Phaseolus lunatus*. Phytopathology 57: 1111–1115.

Bell, A. A. 1970. 4-Hydroxybenzaldehyde and vanillin as toxins formed in leaf wound sap of *Phaseolus lunatus*. Phytopathology 60: 161–165.

Bertrand, G. 1906. La vicianine, nouveau glucoside cyanhydrique contenu dans les graines de vesce. Compt. Rend. Hebd. Séances Acad. Sci. 143: 832–834.

——— & L. Rivkind. 1906. Sur la répartition de la vicianine et de sa diastase dans les graines de Légumineuses. Compt. Rend. Hebd. Séances Acad. Sci. 143: 970–972.

Brimer, L., S. B. Christensen, J. W. Jaroszewski & F. Nartey. 1981. Structural elucidation and partial synthesis of 3-hydroxyheterodendrin, a cyanogenic glucoside from *Acacia sieberiana* var. *woodii*. Phytochemistry 20: 2221–2223.

Bruyning, F. & J. van Haarst. 1899. Sur l'acide cyanhydrique des graines du genre *Vicia*. Rec. Trav. Chim. Pay-Bas 18: 468–471.

Burkart, A. 1952. Las Leguminosas Argentinas, 2nd edition. Acme Agency, Buenos Aires.

Butler, G. W. 1965. The distribution of the cyanoglucosides linamarin and lotaustralin in higher plants. Phytochemistry 4: 127–131.

Butterfield, C. S., E. E. Conn & D. S. Seigler. 1975. Elucidation of structure and biosynthesis of acacipetalin. Phytochemistry 14: 993–997.

Chaudhury, D. N. & A. Robertson. 1949. Synthesis of glycosides. Part XIV. The synthesis of gein and of the hexa-acetyl-β-vicianoside of (-)-mandelonitrile believed to be the hexa-acetate of vicianin. J. Chem. Soc. 2054–2057.

Conn, E. E. 1981. Biosynthesis of cyanogenic glycosides. Pp. 183–196 *in* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley & F. Wissing (editors). Cyanide in Biology. Academic Press, New York.

——— & B. R. Maslin. 1982. A preliminary report on cyanogenesis in Australian *Acacia* species. Int. Group for Study of Mimosoideae. Bull. 10: 26–31.

———, ———, S. Curry & M. E. Conn. 1985. Cyanogenesis in Australian species of *Acacia*. Surveys of herbarium specimens and living plants. West. Austral. Herbarium Res. Notes 10: 1–60.

Corkill, L. 1942. Cyanogenesis in white clover (*Trifolium repens* L.) V. The inheritance of cyanogenesis. New Zealand J. Sci. Technol. 23B: 178–193.

Couperot, E. V. 1913. Recherches sur la présence des azotaes dans les plantes médicinales et alimentaires. Thése (Pharm.), Université Paris.

Cugudda, E., C. Gigli & S. Massenti. 1953. Patogenesi del favismo. Minerva Med. 441: 140–147.

da Costa Sacco, J. 1957. Observações sobre *Sesbania punicea* (Cav.) Benth. Bol. Tecn. Inst. Agron. S. 17: 13–21. [Chem. Abs. 55: 16689 (1961).]

de Peralta, F. 1928. Third list of cyanophoric plants of Maquiling Region. Philipp. Agric. 17: 333–335.

de Waal, H. L. 1939. Chemical investigations upon *Lotononis laxa* E. and Z. I. The Isolation of pinitol, a fatty ester and benzaldehyde. Onderstepoort J. Vet. Sci. Anim. Ind. 13: 229–233.

de Wildeman, E. 1939. Notes sur des plantes médicinales et alimentaires du Conge Belge, Mém. Inst. Roy. Colon. Belge, Sect. Sci. Nat. 9: 115–134.

Dillemann, G. 1958. Composés cyanogénétiques. Pp. 1050–1075 *in* W. Ruhland (editor), Handbuch der Pflanzenphysiologie, Volume 8. Springer-Verlag, Berlin.

Dominguez, J. A., J. F. Molino & E. L. de Gallelli. 1928. Composición quimica de las plantas argentinas. Trab. Inst. Bot. Farmacol. (Buenos Aires). 40: 1–75.

Dunstan, W. R. & T. A. Henry. 1900. The nature and origin of the poison of *Lotus arabicus*. Preliminary Notice. Proc. Roy. Soc. Lond. 67: 224–226.

_____ & _____. 1901. The nature and origin of the poison of *Lotus arabicus*. Phil. Trans. Ser. B 194: 515–533.

_____ & _____. 1903 [1904]. Cyanogenesis in plants. Part III. On phaseolunatin, the cyanogenetic glucoside of *Phaseolus lunatus*. Proc. Roy. Soc. London 72: 285–294. [The volume is dated 1904.]

_____ & _____. 1906 [1907]. The chemical aspects of cyanogenesis in plants. Annual Rep. Brit. Assoc. Adv. Sci. 1906: 145–157. [Printed in 1907.]

Ebinger, J. E. & D. S. Seigler. 1987. Cyanogenic glycosides in ant-acacias of Mexico and Central America. Southwestern Naturalist 32: 499–503.

Egorov, A. D., V. P. Savarin & T. S. Zhilyaeva. 1965. Prussic acid in some food plants in central Yakutia (Russian). Introduktsiya Rast. v. Tsentr. Yakutia (Moscow-Leningrad Nauka) Sb. 194–198. [Chem. Abs. 64: 11550. 1966.]

Ettlinger, M. G., J. W. Jaroszewski, S. R. Jensen, B. J. Nielsen & F. Nartey. 1977. Proacacipetalin and acacipetalin. Chem. Comm. 952–953.

Everist, S. L. 1964. A review of the poisonous plants of Queensland. Proc. Roy. Soc. Qld. 74: 1–20.

Eyjólfsson, R. 1970. Recent advances in the chemistry of cyanogenic glycosides. Fortschr. Chem. Org. Naturst. 28: 74–108.

Feigl, G. & V. Anger. 1968. Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. Analyst 91: 282–284.

Fikenscher, L. H. & R. Hegnauer. 1981. Die Verbreitung der Blausäure bei den Cormophyten. 18. Mitteilung. Weitere Beobachtungen bei den Leguminosae–Papilionoideae (Galegeae, Genisteae, Loteae, Phaseoleae). Pl. Med. 43: 323–335.

Finnemore, H. & J. M. Cooper. 1938. The hydrocyanic acid content of samples of *Lotus australis* and *Heterodendron oleaefolium*. Austral. Vet. J. 14: 153–157.

_____ & C. B. Cox. 1928. Cyanogenetic glucosides in Australian plants. J. Proc. Roy. Soc. New South Wales 62: 369–378.

_____ & W. C. Gledhill. 1928a. Poison plants: the presence of cyanogenetic glucosides in certain species of *Acacia*. J. Council Sci. Res. Australia 1: 254.

_____ & _____. 1928b. The presence of cyanogenetic glucosides in certain species of *Acacia*. Australas. J. Pharm. 9: 174–178.

_____ & D. K. Large. 1936. Cyanogenetic glucosides in Australian plants. Part 6.—an unstable cyanogenetic constituent in *Goodia lotifolia*. Proc. Roy. Soc. New South Wales 70: 440–451.

_____, J. M. Cooper & J. H. Cob-Croft. 1938a. The cyanogenetic constituents of Australian and other plants. Part VII: white clover *(Trifolium repens)*. J. Soc. Chem. Ind. 57: 166–167.

_____, _____ & M. B. Stanley. 1938b. The cyanogenetic constituents of Australian and other plants. Part VII: *Lotus australis*. J. Soc. Chem. Ind. 57: 162–166.

Foley, H. & L. Musso. 1925. Les plantes du Sahara toxiques pour les animaux. Presence d'un glucoside cyanhydrique dans le *Lotus jolyi* Battandier. Arch. Inst. Pasteur Algérie 3: 394–400.

Foulds, W. 1982. Polymorphism for cyanogenesis in *Lotus australis* Andr. populations at Greenough Front Flats, Western Australia. Austral. J. Bot. 30: 211–217.

Freise, F. W. 1938. Zusammensetzung der Samen von *Cajanus* sp. und *Canavalia* sp. zwei brasilianischen Volksnahrungsmitteln. Z. Untersuchung der Lebensmittel 75: 566–568.

Gard, M. 1915. Chimie végétal. Un genre de Légumineuses–Papilionacées nouveau pour le cyanogenèse (genre *Ornithopus* L.). Compt. Rend. Hebd. Séances Acad. Sci. 161: 10–11.

Gardner, C. A. & H. W. Bennetts. 1956. The Toxic Plants of Western Australia. Western Australia Newspapers Ltd., Perth.

Gibbs, R. D. 1974. Chemotaxonomy of Flowering Plants. McGill-Queens Univ. Press, Montreal.

Gibson, P. B., O. W. Barnett & J. T. Gillingham. 1972. Cyanoglucoside and hydrolyzing enzyme in species related to *Trifolium repens*. Crop Sci. (Madison) 12: 708–709.

Giusti, L. 1937 [1938]. Plantas forrajeras cianogenéticas. Jornadas Agron. y Vet. 1937: 43–49.

Gmelin, R., M. Schüler & E. Bordas. 1973. Holocalin: Ein Neues Cyanogenes Glykosid aus *Holocalyx balansae*. Phytochemistry 12: 457–461.

Grant, W. F. & B. S. Sidhu. 1967. Basic chromosome number, cyanogenetic glucoside variation, and geographic distribution of *Lotus* species. Canad. J. Bot. 45: 639–647.

Greshoff, M. 1906a. Sur la distribution de l'acide cyanhydrique dans le règne végétal. Bull. Sci. Pharm. 13: 589–602.

———. 1906b [1907]. The distribution of prussic acid in the vegetable kingdom. Rep. 76th Meet. Brit. Assoc. Adv. Sci. 137–144.

———. 1909. Phytochemical investigations at Kew. Bull. Misc. Inform. 1909: 397–418.

Guérin, P. 1928. Chemie végétal. L'acide cyanhydrique chez les *Lotus*. Compt. Rend. Acad. Sci. Hebd. Séances 187: 1158–1160

———. 1929. Lés Papilionacées–Lotées à acide cyanhydrique. Compt. Rend. Acad. Sci. Hebd. Séances Paris 189: 115–116.

———. 1930. Les Légumineuses à acide cyanhydrique. Bull. Soc. Chim. Biol. 12: 1338–1354.

Guignard, L. 1906. Le haricot à acide cyanhydrique (*Phaseolus lunatus* L.). Etude historique, botanique et chimique. Nouveau procédé pour déceler l'acide cyanhydrique. Bull. Sci. Pharm. 13: 129–138, 193–213, 337–352, 401–419.

———. 1907. Sur les quantités d'acide cyanhydrique formé par le *Phaseolus lunatus* L. cultivé sous le climat de Paris. Bull. Sci. Pharm. 14: 565–569.

Hanelt, P. & B. Tschiersch. 1967. Blausäureglykoside—Untersuchungen am Gaterslebener Wickensortiment. Kulturpflanze 15: 85–96.

Harris, J. R., G. H. J. Merson, M. J. Hardy & D. J. Curtis. 1980. Cyanide in animal feeding stuffs. Analyst 105: 974–980.

Hegnauer, R. 1958. Over de Verspreiding van Blauwzuur bij Vaatplanten. Pharm. Weekbl. 93: 801–848.

———. 1959. Die Verbreitung der Blausäure bei der Cormophyten. 3. Mitteilung. Die Blausäurehaltigen. Pharm. Weekbl. 94: 248–262.

———. 1961. Die Verbreitung der Blausäure bei den Cormophyten. 4. Mitteilung: Neue Untersuchungen über die Verbreitung der Cyanogenese. Pharm. Weekbl. 96: 577–596.

———. 1962–1973. Chemotaxonomie der Pflanzen, Volumes 1–6. Birkhäuser Verlag, Basel.

———. 1971. Probleme der Chemotaxonomie, erläutert am Beispiel der cyanogenen Pflanzenstoffe. Pharm. Acta Helv. 46: 585–601.

———. 1977. Cyanogenic compounds as systematic markers in Tracheophyta. Pp. 191–209 *in* K. Kubitzki (editor), Plant Systematics and Evolution, Supplementum 1. Springer, Vienna.

———. 1986. Chemotaxonomie der Pflanzen, Volume 7. Birkhäuser Verlag, Basel.

Herbert, D. A. 1922. Cyanophoric plants of the Makiling Region. Philipp. Agric. 11: 11–16.

Honig, D. H., M. E. Hockridge, R. M. Gould & J. J. Rackis. 1983. Determination of cyanide in soybeans and soybean products. J. Agric. Food Chem. 31: 272–275.

Hösel, W., J. Berlin, T. N. Hanzlik & E. E. Conn. 1985. In-vitro biosynthesis of 1-(4′-hydroxyphenyl)-2-nitroethane and production of cyanogenic compounds in osmotically stressed cell suspension cultures of *Eschscholtzia californica* Cham. Planta 166: 176–180.

Hughes, M. A. 1981. The genetic control of plant cyanogenesis. Pp. 495–508 *in* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley & F. Wissing (editors), Cyanide in Biology. Academic Press, New York.

Hurst, E. 1942. The Poison Plants of New South Wales. Poison Plants Committee of New South Wales, Sydney.

Jaffé, W. G. 1950. Estudio sobre la inhibición del crecimiento de ratas causada por algunas semillas de leguminosas. Acta Ci. Venez. 1: 62–64.

Janzen, D. H., S. T. Doerner & E. E. Conn. 1980. Seasonal constancy of intra-population variation of HCN content of Costa Rican *Acacia farnesia*. Phytochemistry 19: 2022–2023.

Jones, D. A. 1981. Cyanide and coevolution Pp. 509–516 *in* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley & F. Wissing (editors), Cyanide in Biology. Academic Press, New York.

Juliano, J. B. 1923. Additional cyanophoric plants of the Makiling Region. Philipp. Agric. 11: 231–232.

———. 1933. Additional cyanophoric plants of the Maquiling Region: IV. Philipp. Agric. 22: 254–257.

——— & M. Guerrero. 1935. Cyanophoric plants of the Maquiling Region: V. Philipp. Agric. 24: 22–26.

Kalaw, M. M. & F. M. Sacay. 1925. Some alleged Philippine poison plants. Philipp. Agric. 14: 421–427.

Kaplan, M. A. C., M. R. Figueiredo & O. R. Gottlieb. 1983. Variation in cyanogenesis in plants with season and insect pressure. Biochem. Syst. Ecol. 11: 367–370.

Kingsbury, J. M. 1964. Poisonous Plants of the U. S. and Canada. Prentice Hall, Englewood Cliffs, New Jersey.

Lambert, J. L., J. Ramasamy & J. V. Paukstelis. 1975. Stable reagents for the colorimetric determination of cyanide by modified König reactions. Analytical Chem. 47: 916–918.

Leather, J. W. 1906. Cyanogenesis in plants. Agric. J. India 1: 220–225.

Lucas, B. & A. Sotelo. 1984. A simplified test for the quantification of cyanogenic glycosides in wild and cultivated seeds. Nutr. Rep. Int. 29: 711–719.

Machado, O. & P. Occhioni. 1943. Contribução ao estudo das plantas cianogênicas do Brasil. Rodriguésia 7: 35–44.

Maslin, B. R. & M. Bennett. 1985. Preliminary observations on the cyanogenic properties of two Western Australian *Acacia* species. West. Austral. Herbarium Res. Notes. 10: 61–65.

———, E. E. Conn & J. E. Dunn. 1985. Cyanogenesis in *Acacia pachyphloia*. Phytochemistry 24: 961–963.

———, J. E. Dunn & E. E. Conn. 1988. Cyanogenesis in Australian species of *Acacia*. Phytochemistry 27: 421–428.

Melville, J. & B. W. Doak. 1940. Cyanogenesis in white clover (*Trifolium repens* L.). II. Isolation of the glucosidal constituents. New Zealand J. Sci. Technol. 22B: 67–71.

Mirande, M. 1909. Influence exercée par certaines vapeurs sur la cyanogenèse végétale. Procédé rapide pour la recherche des plantes à acide cyanhydrique. Compt. Rend. Hebd. Séances Acad. Sci. 149: 140–142.

———. 1913. Sur quelques plantes nouvelles à acide cyanhydrique. Compt. Rend. Hebd. Séances Ménj Soc. Biol. 75: 434–435.

Montgomery, R. D. 1964. Observations on the cyanide content and toxicity of tropical pulses. W. Indian Med. J. 13: 1–11.

———. 1965. The medical significance of cyanogen in plant foodstuffs. Amer. J. Clin. Nutr. 17: 103–113.

Moyer, B. G., P. E. Pfeffer, J. L. Moniot, M. Shamma & D. L. Gustine. 1977. Corollin, coronillin, and coronarian: three new 3-nitropropanoyl-D-glucopyranoses from *Coronilla varia*. Phytochemistry 16: 375–377.

Nahrstedt, A. 1976. Prunasin in *Holocalyx balansae*. Phytochemistry 15: 1983–1984.

———. 1981. Isolation and structure elucidation of cyanogenic compounds Pp. 145–181 *in* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley & F. Wissing (editors), Cyanide in Biology. Academic Press, New York.

———, N. Erb & H. Zinsmeister. 1981. Methods of liberating and estimating hydrocyanic acid from cyanogenic plant material. Pp. 461–471 *in* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley & F. Wissing (editors), Cyanide in Biology. Academic Press, New York.

Nartey, F., L. Brimer & S. B. Christensen. 1981. Proacaciberin, a cyanogenic glycoside from *Acacia sieberiana* var. *woodii*. Phytochemistry 20: 1311–1314.

Oke, O. L. 1964. Hydrocyanic acid content and nitrogen-fixing ability of guar. Nature 204: 405–406.

Pammel, L. H. 1911. A Manual of Poisonous Plants, Part 1 (1910), Part 2 (1911). The Torch Press, Cedar Rapids, Iowa.

Petrie, J. M. 1912. Hydrocyanic acid in plants. Part 1. Its distribution in the Australian flora. Proc. Linn. Soc. New South Wales 37: 220–234.

Plouvier, V. 1974. Hétérosides cyanogénétiques et enzymes: le dhurroside du *Macadamia ternifolia* F. Muell. (Protéacées), l'acide cyanhydrique des *Dorycnium* (Légumineuses), la repartition de la linamarase. Compt. Rend. Hebd. Séances Acad. Sci. 279: 1689–1692.

Polhill, R. M. 1981. Papilionoideae. Pp. 191–208 *in* R. L. Polhill & R. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

——— & P. H. Raven. 1981. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

Puymaly, A. 1912. L'acide cyanhydrique dans les plantes vertes. Univ. Bordeaux, Fac. Med. et Pharm. 131: 1–142.

Quisumbing, E. 1949. Vegetable poisons of the Philippines. Philipp. J. Forest. 5: 145–171.
Rehr, S. S., P. P. Feeny & D. H. Janzen. 1973. Chemical defence in Central American non-ant-acacias. J. Anim. Ecol. 42: 405–416.
Rigg, T., H. O. Askew & E. B. Kidson. 1933. Occurrence of cyanogenetic glucosides in Nelson pasture plants. New Zealand J. Sci. Technol. 15: 222–227.
Rimington, C. 1935. The occurrence of cyanogenetic glucosides in South African Species of *Acacia*. II. Determination of the chemical constitution of acacipetalin. Its isolation from *Acacia stolonifera*, Burch. Onderstepoort J. Vet. Res. 5: 445–464.
Ritthausen, H. & U. Kreusler. 1870. Ueber Vorkommen von Amygdalin und eine neue dem Asparagin ähnliche Substanz in Wickensamen *(Vicia sativa)*. J. Prakt. Chem. 110 (new series: 2): 333–338.
Robinson, M. E. 1930. Cyanogenesis in plants. Biol. Rev. (London) 5: 126–141.
Rosenthaler, L. 1919. Beiträge zur Blausäure-Frage. Die Verbreitung der Blausäure im Pflanzenreich. Schweiz. Apoth. Z. 57: 279–283, 295–297, 307–313, 324–329, 341–346.
———. 1923. Zur Prüfung der Treubschen Hypothese. Biochem. Z. 134: 215–224.
———. 1928. Beiträge zur Blausäurefrage. Pharm. Acta. Helv. 3: 31–34.
———. 1929. Beiträge zur Blausäurefrage. Pharm. Acta Helv. 4: 196–199.
Saupe, S. G. 1981. Cyanogenic compounds and angiosperm phylogeny. Pp. 80–116 *in* D. A. Young & D. S. Seigler (editors), Phytochemistry and Angiosperm Phylogeny. Praeger, New York.
Schmutz, E. M., B. N. Freeman & R. E. Reed. 1968. Livestock Poisoning Plants of Arizona. Univ. Arizona Press, Tucson, Arizona.
Secor, J. B., E. E. Conn, J. E. Dunn & D. S. Seigler. 1976. Detection and identification of cyanogenic glucosides in six species of *Acacia*. Phytochemistry 15: 1703–1706.
Seifert, P. 1955. Blausäure-Verbindungen. Pp. 676–688 *in* K. Paech & M. V. Tracey (editors), Moderne Methoden der Pflanzenanalyse, Volume 4. Springer-Verlag, Berlin.
Seigler, D. S. 1976b. Plants of Oklahoma and Texas capable of producing cyanogenic compounds. Proc. Okla. Acad. Sci. 56: 95–100.
———. 1977. The naturally occurring cyanogenic glycosides. Pp. 83–120 *in* L. Reinhold, J. B. Harborne & T. Swain (editors), Progress in Phytochemistry, Volume 4. Pergamon, Oxford.
———. 1981. Recent developments in the chemistry and biology of cyanogenic glycosides and lipids. Rev. Latinoamer. Quim. 12: 39–48.
——— & E. E. Conn. 1982. Cyanogenesis and systematics of the genus *Acacia*. Bull. Int. Group Study of Mimosoideae 10: 32–43.
——— & J. E. Ebinger. 1988. *Acacia macracantha*, *Acacia pennatula*, and *Acacia cochliacantha* species complexes in Mexico. Syst. Bot. 13: 7–15.
———, J. D. Coussio & R. V. D. Rondina. 1979b. Cyanogenic plants from Argentina. J. Nat. Prod. 42: 179–182.
———, J. E. Dunn & E. E. Conn. 1976. Acacipetalin in *Acacia constricta* from North America. Phytochemistry 15: 219–220.
———, C. S. Butterfield, J. E. Dunn & E. E. Conn. 1975. Dihydroacacipetalin. A new cyanogenic glucoside from *Acacia sieberiana* var. *woodii*. Phytochemistry 14: 1419–1420.
———, E. E. Conn, J. E. Dunn & D. H. Janzen. 1979a. Cyanogenesis in *Acacia farnesiana*. Phytochemistry 18: 1389–1390.
———, J. E. Dunn, E. E. Conn & G. L. Holstein. 1978. Acacipetalin from six species of *Acacia* of Mexico and Texas. Phytochemistry 17: 445–446.
———, ———, ——— & J. F. Pereira. 1983. Cyanogenic glycosides from four Latin American species of *Acacia*. Biochem. Syst. Ecol. 11: 15–16.
Sillans, R. 1952. Sur quelques plantes ictyotoxiques de l'Afrique centrale. Rev. Int. Bot. Appl. Agric. Trop. 32: 54–65.
Stermitz, F. R. 1977. Chemical constituents of the Astragaleae (Leguminosae). Rev. Latinoamer. Quim. 8: 1–10.
Steyn, D. G. 1929. 15th Annual Report. Director Veterinary Service, Union of South Africa, Volume II. Section VI.
———. 1934a. The toxicology of plants in South Africa. South African Agric. Ser. XIII. Central News Agency, South Africa.
———. 1934b. The camel-thorn tree and other *Acacia* species as stockfeed. Farming in South Africa 9: 89–90, 106.

———. 1937. Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa. Onderstepoort J. Vet. Sci. Anim. Ind. 9: 573–582.

——— & C. Rimington. 1935. The occurrence of cyanogenetic glucosides in South African species of *Acacia*. I. Onderstepoort J. Vet. Sci. 4: 51–63.

Tantisewie, B., H. W. L. Ruijgrok & R. Hegnauer. 1969. Die Verbreitung der Blausäure bei den Cormophyten. 5. Mitteilung: Ueber Cyanogene Verbindungen bei den Parietales und bei einigen Weiteren Sippen. Pharm. Weekbl. 104: 1341–1355.

Tapper, B. A. & P. F. Reay. 1973. Pp. 447–476 *in* G. W. Butler & R. W. Bailey (editors), Chemistry and Biochemistry of Herbage, Volume 2.

Teik. 1951. Sci. Ser. Dept. Agric. Malaya. 24: 15, 67, 76, 82. [Cited in the Wealth of India.]

Treub, M. 1904. Nouvelles recherches sur le role de l'acide cyanhydrique dans les plantes vertes. Ann. Jard. Bot. Buitenzorg. 2^{e} Sér. 4, Vol. 19: 86–145. [This is cited as 1905 in many publications.]

———. 1907. Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. Ann. Jard. Bot. Buitenzorg. 2^{e} Sér. 6, Vol. 21: 79–106.

Tschiersch, B. & P. Hanelt. 1967. Die freien Aminosäuren der Samen von *Vicia* L. und die systematische Gliederung der Gattung. Flora, Abt. A. 157: 389–406.

Tson Sie Fat, L. A. 1979. Contribution to the knowledge of cyanogenesis in angiosperms. Ph.D. dissertation. Rijks Universiteit te Leiden.

Univ. Arizona Agric. Expt. Stat., Ann. Rep. 1934. *Cercocarpus* and *Acacia* species lethal by HCN 45: 44.

van Romburgh, P. 1899. Notices Phytochimiques. Ann. Jard. Bot. Buitenzorg 16: 1–16.

van Valen, F. 1979. Contribution to the knowledge of cyanogenesis in angiosperms. 13. Communication. Cyanogenesis in some Papilionaceae. Pl. Med. 35: 141–149.

Vennesland, B., E. E. Conn, C. J. Knowles, J. Westley & F. Wissing. 1981. Cyanide in Biology. Academic Press, New York.

Viehoever, A. 1940. Edible and poisonous beans of the lima type (*Phaseolus lunatus* L.) Thai. Sci. Bull. 2: 1–99.

Webb, L. J. 1949. An Australian phytochemical survey 1. Alkaloids and cyanogenetic compounds in Queensland Plants. Bull. 241: 1–56. CSIRO, Australia.

Wokes, F. & S. G. Willimot. 1951. The determination of cyanide in seeds. J. Pharm. Pharmacol. 3: 905–917.

INTERACTIONS BETWEEN ANTS AND LEGUMINOUS PLANTS

Doyle McKey[1]

Abstract

Ants interact with leguminous plants in two ways: as seed dispersers and as a biotic defense against herbivores. Legumes with seeds adapted for ant-dispersal occur in all three subfamilies, but are most frequent in Australian *Acacia* and in the Papilionoideae. Myrmecochorous legumes occur most frequently in habitats that are fire-prone and characterized by nutrient-poor soil and a large proportion of bare ground. In such habitats, seed burial by ants provides protection against seed predation during seed storage until postfire germination, and may place seeds in relatively nutrient-rich microsites. Myrmecochorous legumes are best documented in mediterranean shrublands of Australia and southern Africa, but also occur in European heathlands and in fire-prone tropical and subtropical habitats in the Americas, Africa, and Asia. Protective interactions may be facultative or obligate. In the legumes, facultative interactions involve foraging ants attracted to extrafloral nectaries. Extrafloral nectaries are documented in all three subfamilies, but their frequency varies greatly among taxonomic groups. In the Mimosoideae, they are widespread in the Mimoseae and virtually universal in the Parkieae, Acacieae, and Ingeae. In the Caesalpinioideae, they are widespread in two genera of Cassieae, documented in a few members of Detarieae and Amherstieae, and apparently rare or absent in other groups. In the Papilionoideae, they appear to be rare or absent in most groups, but common in some subtribes of the Phaseoleae and in the genera *Sesbania* (and its ally *Glottidium*), *Vicia* (subg. *Vicia*), *Lotus*, and *Hippocrepis*. In short-lived legumes, nectaries function primarily in protection of reproductive parts, whereas in long-lived woody legumes, ants attracted to nectaries primarily protect young leaves. In woody legumes, nectaries are most common in groups that produce young leaves relatively continuously, and rare in groups that produce young leaves intermittently in large flushes. This is an outcome of the conflict between traits required for phenological defense of young leaves and traits necessary for effective biotic defense. Protective interactions are more species-specific, and often obligate for the plant and/or ants, in plants that possess specialized structures in which ants consistently nest. Myrmecophytes are documented in all three legume subfamilies. In the Mimosoideae and Caesalpinioideae, they have evolved from plants engaged in facultative interactions with foraging ants attracted to extrafloral nectaries; in the Papilionoideae the nature of the facultative interactions that evolutionarily preceded ant-plant symbioses is unclear. Myrmecophytes have evolved in only a few of the many groups of legumes engaged in facultative interactions with ants. One hypothesis suggests that this is largely because preadaptations for ant-domatia in leguminous plants are rare.

I. Introduction

Objectives

The objective of this study is to describe and interpret the radiation of traits related to interaction with ants in one of the largest families of flowering plants. Among the angiosperms, the legumes are probably rivaled only by the Euphorbiaceae in the diversity of ecological interactions with ants and the number of species involved. A survey of these interactions in the Leguminosae must be imperfect, given the current state of information. In addition to presenting and interpreting patterns that are evident from existing information, I emphasize open questions and new hypotheses for which empirical data are sorely needed.

This study is aimed at two groups of scientists. First, for the morphologists and systematists of Leguminosae, I have included discussions of the taxonomic distribution of plant traits related to

[1]Department of Biology, University of Miami, P. O. Box 249118, Coral Gables, Florida 33124, U.S.A.

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interactions with ants. For some of these traits, notably extrafloral nectaries, there are many gaps in descriptive information that render an assessment of their taxonomic distribution problematical. I have delineated the problems of distinguishing ant-attracting nectaries and other glands, and pointed to groups where more information is needed.

Second, for ecologists who study interactions of ants with particular legume species, I included a broadbrush perspective that complements their detailed studies; for ecologists who study interactions between ants and plants of other families, I offer a base for comparison.

SCOPE AND ORGANIZATION

Ants may interact with leguminous plants in two ways: as seed dispersers and as a biotic defense mechanism. The first part of this paper deals with seed dispersal, while the remainder treats the ecological significance of ants that forage and/or nest in legume plants. Although in other families, plants gain a nutritional reward from ants nesting within them, it appears that no legume species is myrmecotrophic (fed by ants). Leguminous myrmecophytes, plants possessing specialized structures (myrmecodomatia) inhabited by ant nests, benefit by ant protection and often host one or a few ant species, the interaction being obligate for ant and plant. Legume species that attract foraging ants without providing ant domatia may also benefit by ant protection, but tend to interact facultatively with a greater variety of ant species.

Extrafloral nectaries are the most conspicuous trait of legume species involved in facultative interactions with opportunistically foraging ants. However, other rewards may also be present. In some plant families (Davidson et al., 1989), trichomes and other superficial structures may influence ant-plant interactions not by offering a nutritional reward, but by affecting the outcome of competition among ant species. The absence of information on the roles of traits other than extrafloral nectaries in facultative interactions of leguminous plants with ants is a shortcoming of this review and an opportunity for further work.

Legume myrmecophytes have been subject to uneven attention. The family includes the best-studied ant-plants of all, the neotropical ant-acacias, as well as some for which only anecdotal information is available. In the final section of the paper, I summarize what is known about legume myrmecophytes, emphasizing what little literature exists on several poorly known ant-plants.

II. Dispersal of Legume Seeds by Ants

Seeds of some legumes (e.g., *Ulex* species, *Cytisus scoparius*) have long been known to be dispersed by ants (Sernander, 1906; Weiss, 1908, 1909). Likewise, morphologists have known that many Papilionoideae possess seed appendages similar to those that function as elaiosomes in *Ulex* and *C. scoparius* (see Fig. 1). However, until recently there has been little interest in their role in seed dispersal. For Corner (1949), "rim-arils" and other papilionoid seed appendages were functionless remnants of durianlike glory. Recent work, spearheaded by Berg (1975, 1979), has shown that ants are the principal dispersal agents of many such appendagebearing legume seeds. Berg's work in sclerophyll vegetation of Australia has stimulated further work there (Shea et al., 1979; Drake, 1981; Rice & Westoby, 1981) and in the Cape region of southern Africa, where mediterranean sclerophyll vegetation appears to be another center of diversity of myrmecochorous plants, in legumes as well as in other families (Slingsby & Bond, 1981; Bond & Slingsby, 1983). The realization that seeds adapted for ant-dispersal are common in these dry habitats has added a new dimension to the study of myrmecochorous plants, most of which, until about 10 years ago, were thought to be temperate-forest herbs. Much recent work has focused on testing hypotheses about why adaptations for ant-dispersal are so common in certain dry habitats (Beattie, 1983). Since most legumes known to possess elaiosomes are typical of such habitats, this work is highly relevant to

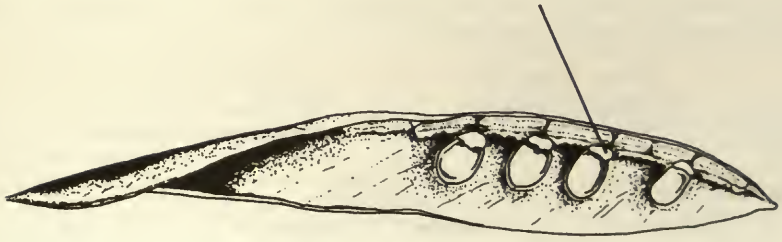

FIGURE 1. Single valve of a mature fruit of *Cytisus scoparius* (Papilionoideae: Genisteae), showing elaiosomes (arrow).

my review. I will summarize what is known about the taxonomic distribution of myrmecochorous seeds in the Leguminosae, review ecogeographic patterns evident from analysis of their distribution, and discuss the results of ecological studies of ant-dispersal of legume seeds. I will also suggest profitable areas for further research.

TAXONOMIC DISTRIBUTION

Table 1 summarizes current information on the taxonomic distribution within the Leguminosae of seeds with adaptations for ant-dispersal. This information must be offered with several caveats. First, information on dispersal is more limited than information on occurrence of putative adaptations for ant-dispersal. Nevertheless, ant-dispersal has been directly observed in species of 15 genera belonging to six papilionoid tribes, and also in *Acacia*. In addition to the 16 genera in which ant-dispersal has been observed, Table 1 lists genera possessing seed appendages that probably function as elaiosomes but have not been studied in the field. Joining Berg (1975) and Beattie (1983), I suspect ant-dispersal will eventually be documented in most of the genera listed in Table 1. My compilation is based on photographs, drawings, or good descriptions of seeds. Berg (1979) reviewed the terminological confusion that makes it difficult to interpret cursory descriptions of legume seed appendages. For example, the term "rim-aril" has been applied by different authors to membranous seed appendages that probably do not function as elaiosomes and to fleshy ones that probably do. This problem has also been discussed by Polhill (1976) and Gunn (1981). Table 1 includes few taxa with membranous appendages lacking a dispersal function, yet the table is probably incomplete. My compilation is based on general treatments. Study of generic monographs and local floras, and examination of a broad range of living plants, would probably add several genera to the list.

Second, my compilation of the occurrence of elaiosomelike arils is at the generic level. In some genera, arils may be present or absent in different species (Polhill, 1971). Table 1 indicates such cases where they are known.

Third, some seeds dispersed primarily by ants possess no obvious elaiosome; rather, ants are attracted by oil impregnated in the testa (Sernander, 1906; Drake, 1981). Though this has not been observed in legumes, it cannot be excluded a priori. Small-seeded legumes lacking arils in habitats rich in myrmecochorous plants may require closer examination. A possible example is *Aspalathus linearis* (Crotalarieae) in southern Africa, seeds of which are collected in large numbers by ants although they lack an elaiosome. This plant is the source of the economically important rooibos tea. Ant nests are dug up to obtain viable seeds for sale (B. E. van Wyk, pers. comm.). According to Morton (1983), a single ant hill has yielded up to 25,000 seeds. The identity of the ants, and whether they act as dispersers or eventually kill *A. linearis* seeds they collect, is apparently unknown.

TABLE 1. Taxonomic distribution of seeds with adaptations for ant dispersal in the Leguminosae. Genera according to treatments in Polhill & Raven (1981).

Subfamily	Tribe	Total number of genera	Number of genera with elaiosomes	Identity	Distribution of elaiosomes within genus*	Ant-dispersal confirmed	References
CAESALPINIOIDEAE							
	Cassieae	20	2	*Labichea*	apparently all species		Irwin & Barneby, 1981
				Petalostylis	apparently all species		Berg, 1975
MIMOSOIDEAE							
	Acacieae	2	1	*Acacia*	ca. 300/500 Australian species	+	Berg, 1975; Drake, 1981; Davidson & Morton, 1984
PAPILIONOIDEAE							
	Millettieae	44	1	*Tephrosia*	ca. 15/23 Australian species		Berg, 1975
	Desmodieae	27	2	*Desmodium*	subg. *Sagotia*		van der Pijl, 1956
				Codariocalyx			Ohashi et al., 1981
	Phaseoleae	84	10	*Ophrestia*	apparently all species		Lackey, 1981
				Pseudo-eriosema	apparently all species		Lackey, 1981
				Cruddasia	apparently all species		Lackey, 1981
				Kennedia	15/15 Australian species	+	Berg, 1975, 1979; Drake, 1981
				Hardenbergia	3/3 Australian species	+	Berg, 1975, 1979; Drake, 1981
				Vandasia (apparently grouped with *Hardenbergia* by Berg, 1975)			
				Atylosia	6/6 Australian species		Berg, 1975
				Chrysoscias			Bond & Slingsby, 1983
				Rhynchosia	2/6 Australian species		Berg, 1975
				Eriosema	apparently all species		Grear, 1970; Grear & Dengler, 1976
	Adesmieae	1	1	*Adesmia*	rarely arillate		Polhill, 1981
	Mirbelieae	23	12	*Brachysema*	ca. 14/14 Australian species		Berg, 1975

TABLE 1. Continued.

Subfamily	Tribe	Total number of genera	Number of genera with elaiosomes	Identity	Distribution of elaiosomes within genus*	Ant-dispersal confirmed	References
				Mirbelia		+	Shea et al., 1979
				Gompholobium			Polhill, 1971; Rice & Westoby, 1986
				Burtonia			Polhill, 1971
				Sphaerolobium			Polhill, 1971
				Daviesia	60/60 Australian species	+	Berg, 1975
				Aotus	1/12 Australian species		Berg, 1975
				Gastrolobium	42/42 Australian species		Berg, 1975
				Pultenaea	6/6 Australian species	+	Berg, 1975
				Latrobea	6/6 Australian species		Berg, 1975
				Eutaxia	15/15 Australian species		Berg, 1975
				Dillwynia	15/15 Australian species	+	Berg, 1975; Rice & Westoby, 1986
	Bossiaeeae	10	8	*Lamprolobium*	1/1 Australian species		Berg, 1975
				Plagiocarpus			Polhill, 1971
				Templetonia	7/7 Australian species		Berg, 1975
				Hovea	14/14 Australian species	+	Berg, 1975
				Bossiaea	40/40 Australian species	+	Berg, 1975; Shea et al, 1979; Drake, 1981; Rice & Westoby, 1986
				Platylobium	3/3 Australian species		Berg, 1975
				Goodia	2/2 Australian species	+	Berg, 1975
				Aenictophyton			Polhill, 1971
	Podalyrieae	3	2	*Cyclopia*			Bond & Slingsby, 1983
				Podalyria		+	Bond & Slingsby, 1983
	Liparieae	5	5	*Hypocalyptus*		+	Bond & Slingsby, 1983
				Liparia			Polhill, 1971
				Priestleya		+	Bond & Slingsby, 1983
				Amphithalea		+	Bond & Slingsby, 1983

TABLE 1. Continued.

Subfamily	Tribe	Total number of genera	Number of genera with elaiosomes	Identity	Distribution of elaiosomes within genus*	Ant-dispersal confirmed	References
				Coelidium			Bond & Slingsby, 1983
	Crotalarieae	16	1	*Crotalaria*	some species of subsect. *Incanae*; typical for sect. *Dispermae*		Polhill, 1968
	Genisteae	20	7	*Cytisophyllum*			Polhill, 1976
				Argyrocytisus			Polhill, 1976
				Chamaecytisus			Polhill, 1976
				Cytisus		+	Polhill, 1976; Weiss, 1909
				Genista	several sections		Polhill, 1976
				Stauracanthus			Polhill, 1976
				Ulex			Weiss, 1909

*Where specifically noted in the references.

Finally, arils may be taken, and the seeds bearing them dispersed, by animals other than ants. Diaspores of some Australian *Acacia* species are taken only by ants, while others, primarily bird-dispersed, may benefit from subsidiary dispersal by ants (Davidson & Morton, 1984). Secondary seed movement by ants, attracted to remains of fleshy fruit adhering to seeds that have passed through vertebrate guts, may also be more common than is reported (cf. Beattie, 1983). Small-seeded ornithochorous *Acacia* and *Pithecellobium* species are likely candidates.

With these qualifications in mind, we can approach the results presented in Table 1. Almost all genera with seeds adapted for dispersal by ants belong to the Papilionoideae. I could find references to only two caesalpinioid genera with elaiosomelike arils, *Petalostylis* (Berg, 1975) and *Labichea* (Irwin & Barneby, 1981), the sole members of the subtribe Labicheinae (tribe Cassieae). These xeromorphic Australian shrubs occur in habitats with many other ant-dispersed plants.

In the Mimosoideae, ant-dispersal has been well documented in Australian species of *Acacia*. According to Berg (1975), approximately 300 of the 500 Australian *Acacia* species appear to be myrmecochorous. In contrast to bird-dispersed Australian *Acacia*, species adapted for ant-dispersal have smaller diaspores and white or off-white arils of comparatively low energy reward (Davidson & Morton, 1984). Berg (1975) noted that some Australian *Acacia* possess a colored funicle in addition to the white aril, and he considered its function unknown. The role of the colored funicle in dispersal of seeds of other *Acacia* species by birds (Glyphis et al., 1981; Davidson & Morton, 1984) suggests that some acacias are adapted to dispersal by both kinds of animals.

Within the Papilionoideae, seeds adapted for ant-dispersal occur in nine of 32 tribes in the treatment of Polhill & Raven (1981). Seeds of a tenth tribe, Brongniartieae, possess small arils, but I have not seen good descriptions of the seeds and arils, including information on diaspore size and other characteristics, that would determine the likelihood of dispersal by ants. In three of the nine tribes known to possess them, elaiosomelike arils are restricted to one or two genera. In the Millettiae, they are documented in only one *(Tephrosia)* of the 44 genera. In the Desmodieae, they appear to occur only in *Desmodium* (subg. *Sagotia*) and in *Codariocalyx*. In the Crotalarieae, they appear only in some species of *Crotalaria*. In other tribes, many or all genera are characterized by elaiosomelike arils. These include Mirbelieae, Bossiaeeae, Podalyrieae, and Liparieae. In the

Mirbelieae, arils have been documented in at least 12 of the 23 genera and probably occur in others. Eight of the ten genera of the Bossiaeeae appear in Table 1. Seeds of the other two genera are exarillate *(Muelleranthus)* or not well known *(Ptychosema)* (Polhill, 1976). Seeds of members of the small tribe Liparieae are apparently always arillate. In the Podalyrieae, two of the three genera possess arillate seeds. In the Phaseoleae, two small subtribes (Kennediinae and Ophrestiinae) are characterized by the presence of arils, and in a third (Cajaninae), they have been documented in four of 13 genera (including one of the largest genera in the subtribe, *Eriosema,* in which they are common). Occurrence of arils has been studied most exhaustively in the Genistae (Polhill, 1971, 1976), where they are present in some genera, yet do not occur in all species.

As discussed in the following section, adaptations for ant-dispersal in the Papilionoideae are most frequent in shrubs and herbs in relatively dry habitats. They are absent in tribes consisting mostly of trees, shrubs, and vines of tropical forests (Swartzieae, Sophoreae, Dipterygeae, Dalbergieae, Abreae, Millettieae, except for *Tephrosia,* which occurs in drier areas than many others of the tribe, and Robinieae). They are also absent or rare in groups comprised largely of herbs or shrubs in mesic temperate-zone habitats (e.g., Galegeae, Vicieae, Trifolieae, Thermopsideae).

Possession of a dehiscent pod seems to be an essential preadaptation for the evolution of an elaiosome among leguminous plants. Tribes with many representatives in mediterranean or dry-tropical habitats, but in which arils are infrequent or lacking, often have indehiscent fruits (e.g., Desmodieae, Psoraleeae, Amorpheae, Aeschynomeneae, Hedysareae, Loteae, Coronilleae), so that an aril would be of little use in attracting ants. Studies should be directed to determining whether any of these, especially those in which the fruit breaks into one-seeded articles, have evolved other ant-attractant features associated with the fruit wall. The glandular fruits of *Cullen* and other Psoraleeae might offer examples (C. Stirton, pers. comm.). It is interesting that in at least two of the seven groups of Desmodieae that have evolved deshiscent fruits (*Codariocalyx* and *Desmodium* subgen. *Sagotia:* Ohashi et al., 1981), arillate seeds are also present.

Why are seeds with elaiosomelike arils more frequent in the Papilionoideae than in the other two subfamilies? Possibly the main reason is that more papilionoid genera occur in dry sites where ant-dispersal offers the greatest selective advantage. The success of this subfamily in such habitats is presumably due to a number of key adaptations. The cases of *Acacia, Petalostylis,* and *Labichea* show that representatives of the other two subfamilies also possess morphological substrates for the evolution of elaiosomes in environments where they confer a selective advantage.

The mosaic distribution of elaiosomelike arils in the Papilionoideae underscores the contention of Polhill (1976) that the aril is an evolutionarily labile trait and Berg's (1979) conclusion that arils adapted for ant-dispersal must have evolved independently many times in the subfamily. Based on his studies of seed development and the ontogenetic origin of papilionoid arils, Berg (1979) contended that all papilionoid seeds possess structures capable of being modified by selection into elaiosomes. According to Berg, papilionoid arils evolved several times independently from the same morphological base, the counterpalisade initial, and thus are an example of parallel evolution. Phrased another way, papilionoid arils are homoiologies, "analogies on an homologous base" (Riedl, 1979).

Berg (1975, 1979) pointed out that the Australian ant-dispersed plants, including legumes, are diplochorous, combining ballistic dispersal (in legumes, usually accomplished by explosive dehiscence of the pod) with subsequent dispersal of the scattered seeds by ants. He argued that adaptations for ant-dispersal arose as legumes with purely ballistic seed dispersal invaded particular habitat types or responded evolutionarily to climatic change.

ECOGEOGRAPHIC PATTERNS IN THE DISTRIBUTION OF ANT-DISPERSED LEGUMES

If adaptations for ant-dispersal did occur repeatedly, this mode of seed dispersal must have conveyed particular selective advantages. An examination of ecogeographic patterns in the distribution of legumes adapted for ant-dispersal suggest what these advantages may be.

Berg (1975) pointed out that in Australia most elaiosome-bearing species are found only in two major vegetation types, dry sclerophyll forest/woodland, and dry heath/shrubland. They are apparently absent from alpine vegetation, rainforests, wet sclerophyll forests, and most grassland communities. Ant-dispersed plants are also common in Australian saltbush shrublands (Davidson & Morton, 1981b), and occur in some kinds of grassland communities (D. Davidson, pers. comm.). Noting the importance of fire as an environmental factor in communities where myrmecochory is common, Berg postulated that seed burial by ants could increase seed survival in at least two ways in fire-prone habitats. First, burial protects seeds against high temperatures at the soil surface during fires. Second, in these habitats seedling establishment is virtually confined to the period immediately following a fire (Berg, 1975; Shea et al., 1979; Bond & Slingsby, 1984). Seeds produced during interfire periods must be stored, and burial reduces mortality (due to seed predators and other causes) of stored seeds until the next fire.

However, fire does not seem to be the only environmental factor influencing the frequency of ant-dispersed plants. Fire-prone mediterranean vegetation in Europe and California is poor in ant-dispersed species (Mueller, 1933; Berg, 1966). Berg (1981) noted that the Australian habitats where ant-dispersal is common are also characterized by nutrient-poor soils. Further work has documented the association of nutrient-poor soils and high frequency of ant-dispersed plants, both in Australia (Davidson & Morton, 1981a, b, 1984; Drake, 1981; Westoby et al., 1982) and in the southwestern Cape Province in southern Africa (Slingsby & Bond, 1981; Milewski, 1982; Milewski & Bond, 1982; Bond & Slingsby, 1983), where the high frequency of ant-dispersed species, including several papilionoids, in fynbos vegetation presents a striking example of ecological convergence with similar vegetation in Australia. Interestingly, Slingsby & Bond (1981) and Bond & Slingsby (1983) found elaiosome-bearing species to be common in fynbos, a heathland formation occurring on phosphorus-deficient soils, but rare in adjacent shrublands on nutrient-rich soils.

Two hypotheses, not mutually exclusive, have been proposed to explain the high frequency of ant-dispersed plants in vegetation on nutrient-poor soils. First, in habitats with generally poor soil quality, selection may favor plants that direct their seeds to nutrient-rich microhabitats such as nest mounds of many ants (Beattie, 1983; Davidson & Morton, 1984). The nutrient-enrichment hypothesis was not supported in the one study in which it was tested experimentally (Horvitz & Schemske, 1986). In this study of *Calathea ovandensis* (Marantaceae) in a wet tropical forest in Mexico, chemical differences between ant-nest soil and other soil were not as dramatic as those found by Davidson & Morton (1981) in arid Australian habitats where ant-dispersed plant species are frequent. However, in a recent study in nutrient-poor Australian sandstone soils, Rice & Westoby (1986) found no evidence for the nutrient-enrichment hypothesis in these habitats. Though these studies do not support the hypothesis, the data presented are not sufficient to reject it.

Second, elaiosomes may ensure seed dispersal at a lower nutrient cost (e.g., phosphorus, potassium: Milewski & Bond, 1982) than that of a fleshy fruit attractive to birds and might thus be favored over fleshy fruits in plants in nutrient-poor sites.

In addition to fire and impoverished soils, there may be a third important common denominator of dry habitats rich in elaiosome-bearing species. A substantial proportion of the surface in these habitats is occupied by bare ground. On this substrate, seed-eating vertebrates such as rodents, doves, and finches can forage efficiently. The result is often high rates of predation of seeds that are not buried (Bond & Slingsby, 1984; Bond & Breytenbach, 1985). The vulnerability of unburied seeds compounds the problem of seed storage until after a fire (or any other event that triggers germination, such as rainfall), and may be an additional reason why adaptations for ant-dispersal are common in some dry habitats. The fact that many elaiosome-bearing legume seeds have mottled seed coats that make them cryptic on sand or gravel substrates is circumstantial evidence for the importance of visually searching seed predators. Scattering of seeds by explosively dehiscent fruits, cryptic coloration, and elaiosomes that remain attractive for many days (Berg, 1975) may increase the probability that seeds survive until discovered by ants.

Elaiosome-bearing legumes that are polymorphic for seed-coat color offer possibilities for testing

this hypothesis. Plants of the genus *Eriosema* are characterized by elaiosomelike seed appendages and are probably ant-dispersed (Grear, 1970; Grear & Dengler, 1976). In *Eriosema parviflorum*, some plants produce black seeds, others produce speckled, greenish yellow seeds (Stirton, 1981a). Interestingly, black seeds are rarely attacked by insects in the pod, while speckled seeds are heavily attacked by bruchid beetles and other insects. This suggests a chemical polymorphism similar to that found by Cook et al. (1971) in *Eremocarpus setigerus* (Euphorbiaceae). These authors found that mottled, cryptic seeds were palatable to doves, while gray seeds were unpalatable. Although in Stirton's (1981a) study palatability to birds was not investigated, his observations suggest that in *Eriosema*, elaiosomes, crypticity, and chemical defense may represent multiple defenses against seed predation.

Beattie (1983) discussed the ecological distribution of ant-dispersed plants and concluded that seed placement in nutrient-rich ant nests, escape from seed predators, avoidance of fire, and other factors may all be important advantages of myrmecochory. Studies that attempt to assess the relative importance of these factors and the ways they interact in particular systems are rare. Of studies involving leguminous plants, that of Davidson & Morton (1981a) on Australian *Acacia* species emphasized the role of nutrient-rich microhabitats for germination, that of Shea et al. (1979) on legumes (including *Acacia* species) in dry sclerophyll forest emphasized fire and seed storage. Rice & Westoby (1986) concluded that an adaptive explanation for the geographic distribution of myrmecochory is still a problem.

Berg (1975, 1979) stated that some African and South American legume species are probably myrmecochorous. Recent work in southern Africa has borne this out. However, little attention is being focused on seed dispersal by ants outside mediterranean Australia and southern Africa. Table 1 includes an attempt to identify ant-dispersed legume taxa in other parts of the world. It is significant that most legume taxa bearing an elaiosomelike aril occupy habitats similar to those of known myrmecochores. *Eriosema* species in the New World typically grow in savannalike areas "characterized by poor sandy soils of various types, an impermeable pan at varying depths beneath the surface, poor drainage, and regular seasonal fires" (Grear, 1970). In *Crotalaria*, well-developed arils occur in some species of subsection *Stipulosae* of section *Incanae*, and generally in section *Dispermae* (Polhill, 1976). Section *Dispermae* consists of ca. 140 species, practically confined to south-central Africa, where it has undergone an immense radiation in deciduous woodlands (Polhill, 1968), such as those dominated by the caesalpinioid trees *Brachystegia* and *Julbernardia*. Fires are frequent in these habitats (White, 1983), and Bell (1982) believed their soils to be poorer in nutrients than other savanna/woodland areas in Africa. *Desmodium*, *Tephrosia*, *Rhynchosia*, and other genera in which seeds sometimes possess elaiosomelike appendages, are typically found in open, dry savannalike, or disturbed habitats. European ant-dispersed legumes (e.g., *Ulex*, *Cytisus scoparius*) occur in open, fire-prone, nutrient-poor heathland communities (van der Pijl, 1972; pers. observ.).

III. Facultative Protective Interactions

Plants may benefit in several ways from the activity of ants on their surfaces. Ants eat eggs, juveniles, and adults of phytophagous insects encountered on plants. They may also eat spores of plant-pathogenic fungi. Their presence can discourage mobile insects from feeding and/or ovipositing on plants. Some ants prune vines and other vegetation contacting plants in which they nest. Many plants have evolved traits whose principal function appears to be attraction of ants. Extrafloral nectaries are the most conspicuous traits attracting these "pugnacious bodyguards" (Bentley, 1977), but several other such traits exist. Like extrafloral nectaries, pearl bodies, lipidfilled structures avidly harvested by ants, are widely distributed in many plant families and are important in facultative interactions with ants (O'Dowd, 1982). These do not seem to be common in the

Leguminosae, having been noted so far in only two legume genera, *Bauhinia* and *Vicia.* It is possible that they are more widespread in the family.

Extrafloral nectaries have been documented in many Leguminosae. In addition to well-characterized nectaries, legumes possess a diversity of glandular hairs, glands, and similar structures on the surfaces of leaves and other organs, in which the chemical nature of the contents is unknown (Metcalfe & Chalk, 1972), and whose ecological significance is a mystery (Stirton, 1981b). Perhaps some of these, like nectaries, play a significant role in interactions with ants.

ECOLOGICAL STUDIES

Of the large number of legume genera known to possess extrafloral nectaries, in only five (excluding myrmecophytes, which will be discussed in part IV) have there been quantitative studies of interactions with ants: *Chamaecrista* (Barton, 1986) in the Caesalpinioideae; *Acacia* (Majer, 1979; Buckley, 1983) and *Inga* (Koptur, 1982, 1984, 1985) in the Mimosoideae; and *Vicia* (Koptur, 1979, 1986; Koptur & Lawton, 1988) and *Erythrina* (Feinsinger et al., 1979; Sherbrooke & Scheerens, 1979) in the Papilionoideae. *Acacia* and *Inga* possess nectaries on the leaf rachis, *Chamaecrista* and *Vicia* have stipular nectaries, and *Erythrina* possesses nectaries on stipules and stipels, with structurally similar nectaries on the calyx. Studies of the first four genera have demonstrated experimentally that ants attracted to extrafloral nectaries protect plants from insect herbivores. In *Chamaecrista fasciculata* (Barton, 1986), *Acacia decurrens* (Buckley, 1983) and *Vicia* species (Koptur, 1979), decreased damage leads to increased plant growth and seed set. The *Inga* species studied by Koptur (1984) are large rainforest trees; the protective effects of ants she demonstrated with saplings would be expected to affect growth rates and probability of survival to reproductive maturity. Ants attracted to nectaries on the calyces of floral buds of *Erythrina* protect the inflorescences from dipteran and lepidopteran larvae (Feinsinger et al., 1979).

These studies illustrate several features common to many facultative protective ant-plant interactions. First, nectar secretion and ant activity vary over time. As in other legumes (Nieuwenhuis, 1907) and in plants in other families (Bentley, 1977), foliar nectaries tend to be most active when the leaves are very young; in *Inga,* production of extrafloral nectar is restricted to new and developing leaves (Koptur, 1984). This pattern of activity suggests that the prime function of foliar nectaries is to provide protection during this vulnerable stage in leaf development. Likewise, extrafloral nectaries on or near reproductive structures often exhibit highest activity during a vulnerable stage in development of flowers and/or fruits (Bentley, 1977).

Second, the degree of protection varies depending on abundance of ants and herbivores, proximity of plants to ant colonies, ant species attracted, the kinds of herbivores present, and habitat. Barton (1986) found that the effects of ants visiting extrafloral nectaries of *Chamaecrista fasciculata* varied greatly among populations. Only where both ants and potential herbivores were abundant did ants attracted to nectaries strongly increase plant reproductive success. Koptur (1986) and Koptur & Lawton (1988) found that ants attracted to nectaries of *Vicia sativa* were effective against most externally feeding insect herbivores but not against the endophagous pod-feeding guild. Furthermore, ant activity apparently reduces parasitoid attack of the pod-feeders. Seed production was highest in sites with low ant density where parasitism was higher.

Third, the scope of these facultative mutalisms may also extend to other predaceous and parasitic insects. Koptur (1985) found that *Inga* species at high elevations in Costa Rica had active extrafloral nectaries, even though ant activity was much lower than in lowland habitats. At high elevations, nectaries were visited more by dipteran and hymenopteran parasitoids, and a higher proportion of caterpillars taken from *Inga* were parasitized than at low elevations. Attraction of parasitoids and predators other than ants may also explain the presence of active extrafloral nectaries on a few plants (including three species of *Acacia*) endemic to the Hawaiian Islands, which lack native ants (Keeler, 1985).

The effects on the plant of ants visiting extrafloral nectaries can vary complexly when the ants are accompanied by myrmecophilous phytophagous insects. In the Leguminosae, as in other plant families, these insects are often restricted to host plants bearing extrafloral nectaries, presumably because on such plants ant predictability and abundance are increased and herbivores may gain access to an enemy-free space (Atsatt, 1981). Many myrmecophilous Homoptera (Buckley, 1983) and Lepidoptera (lycaenid caterpillars: Atsatt, 1981) are associated with extrafloral nectary-bearing Leguminosae.

Interactions between ants and myrmecophilous insects in some cases have a net positive effect on the plant, in others a negative effect (Beattie, 1985), depending on whether the damage these insects cause outweighs the indirect benefit they confer by serving as an additional ant attractant. These honeydew-secreting insects have been considered analogous to extrafloral nectaries (Leston, 1973), but there are three important differences between extrafloral nectaries and these biotic "mobile nectaries." First, seen as nectar-producers, they are energetically less efficient than nectaries because the resources taken by myrmecophilous insects are converted largely into products other than honeydew, i.e., into their own growth and reproduction. Second, the plant can exert little direct control on the populations of these insects (and hence over the amount of nectar secreted). Third, the plant also has only limited control over the location of these insects on the plant (hence over the placement of nectar secretion and of ant guards). From the perspective of effects on plant fitness, these "mobile nectaries" may be too mobile.

Buckley (1983) found that ants protected *Acacia decurrens* when honeydew-secreting membracid bugs were absent. When occurring alone, these sapsucking bugs decreased plant growth and seed set. But plants with both bugs and ants fared worse because these bugs, in addition to sucking sap, attracted ants away from the plant's nectaries, decreasing the efficacy of protection by ants.

TAXONOMIC DISTRIBUTION OF EXTRAFLORAL NECTARIES IN THE LEGUMINOSAE

> "This huge and very natural group of plants . . . can be the object of very interesting research. The myrmecophilous function [extrafloral nectaries] is totally absent in many genera and tribes. On the other hand, we can find it developed in a group the size of a subfamily (e.g., among Mimosoideae), the size of a tribe (e.g., among Phaseoleae), the size of a genus (e.g., *Cassia*), or the size of a subgenus (e.g., section *Viciosae* [(= subgen. *Vicia*)] of the genus *Vicia*). . . . But the most important thing to be considered is the various morphological nature of the nectariferous organs; because to each different form, from a historical point of view, corresponds a resurrection of the function" (Delpino 1886, translated by C. Baroni-Urbani).

Many traits of plants are important to interactions with ants that forage and/or nest opportunistically in their crowns. However, the ecology of arboreally foraging and nesting ants is in general poorly understood, and the only kinds of traits that have received much attention in published studies of nonmyrmecophytes are those related to the trophic basis of the interaction. Most of these concern only one type of plant structure that produces food for ants—extrafloral nectaries.

What is an Extrafloral Nectary?

Despite the fair amount of attention that extrafloral nectaries of leguminous plants have received from morphologists and recently from ecologists, there are many uncertainties in interpreting published reports of their occurrence. I will discuss these before turning to an assessment of the taxonomic distribution of extrafloral nectaries within the family.

One source of uncertainty is the variety of plant structures that can function as nectaries (see Figs. 2–6). Extrafloral nectaries are nectar-secreting glands that may occur on any part of the plant other

than the floral nectary. As Bentley (1977) pointed out, even floral nectaries, through postfloral nectar secretion, can function ecologically as extrafloral nectaries, attracting ants that protect developing fruits. To my knowledge, postfloral nectar secretion has never been documented in Leguminosae, though it probably has not been sought out by recent workers. Daumann (1932) noted that Kurr, writing in 1833, suspected it in many Leguminosae. Conversely, extrafloral nectaries in some plants may function in attracting pollinators (Bentley, 1977). Knox et al. (1985, 1986) argued convincingly that attraction of avian pollinators is the primary function of the large, bright red extrafloral nectaries of *Acacia terminalis*.

Not only may nectaries be found on many parts of the plant, they may also assume a wide variety of forms. The petiolar/jugal nectaries of Mimosoideae (see Fig. 2) and of *Senna* and *Chamaecrista* (Cassieae) are conspicuous, raised structures, evident on dried herbarium specimens as well as on living plants. Other kinds of nectaries, e.g., formless nectaries (Zimmermann, 1932) are less conspicuous. These may include nectar-secreting stomates showing no visible modifications. Inconspicuous formless nectaries are apparently frequent on stipules and stipels of Phaseolinae (Zimmerman, 1932) and have recently been found on leaflets of seedlings of *Sesbania* (McKey & Hossaert, unpublished; R. Monteiro, pers. comm.). These nectaries are only evident on close examination of living plants and are likely to be more widespread in the Leguminosae than indicated in the literature.

On the other hand, other structures have often been misinterpreted as extrafloral nectaries. Plants possess a great variety of non-nectariferous glandular structures, but some early workers, including Delpino (1886–1889), were not always critical in distinguishing between these and nectaries (Zimmerman, 1932). Similar confusions persist today. Bhattacharyya & Maheshwari (1971b) listed several caesalpinioid genera as possessing extrafloral nectaries, citing three morphological treatises as authorities (Solereder, 1908; Metcalfe & Chalk, 1950; Leonard, 1957). These authorities, however, mentioned various glandular structures and did not specifically refer to nectary glands, except in the case of *Cassia*. I prefer to discount these records of nectaries until more information is available.

Bhattacharyya & Maheshwari (1971a) described extrafloral nectaries from a variety of Papilionoideae without verifying the composition of any secretion, or indeed whether secretion occurred. Except for the well-known nectaries of *Vicia faba*, I believe the structures they described are referable to the glandular hairs described by authors such as Lackey (1981). The "nature, ontogeny, and function of glands in the Papilionoideae are poorly known and much in need of investigation" (Stirton, 1981b). The bulk of evidence from structure and content of hairs of "gland-dotted" papilionoids argues against their being nectaries (Metcalfe & Chalk, 1972; Turner, 1986). Some of the structures included in Bhattacharyya & Maheshwari's (1971a, b) lists of nectaries (e.g., the pearl glands of *Bauhinia anatomica*) may play a role in interactions with ants; some may turn out to be nectaries. Currently, however, there is little evidence for this.

Some records I consider questionable have been listed in table 2 of Bentley's (1977) valuable review. First, she cites Boehmker (1917) on extrafloral-nectary occurrence in *Acacia, Apuleia, Dialium, Humboldtia, Heterostemon, Macrolobium, Pithecellobium, Tachigali,* and *Vicia*. Boehmker actually lists only *Acacia, Pithecellobium,* and *Vicia*. The others appear to come from the list of Bhattacharyya & Maheshwari (1971b), which may have been inadvertently omitted from her bibliography. Bhattacharyya & Maheshwari (1971a) are cited for *Robinia, Bauhinia,* and *Caesalpinia,* but the latter two are mentioned only in Bhattacharyya & Maheshwari (1971b), and I could find no mention of *Robinia* in either paper. Also, the reference cited for *Pueraria* does not mention this genus.

Other published records I regard as questionable are as follows: *Hoffmannseggia* (recorded with a question mark by Delpino), *Caesalpinia gilliesii* (tiny dark-red punctate glands on pinnule margins: Rippa, 1903; Zimmermann, 1932), and *Delonix regia* (on rachis beneath first pair of pinnae: Zimmermann, 1932) in the Caesalpinieae; *Glycine sinensis* (four dot-shaped glands at leaflet base: Delpino, 1886–1889; Zimmermann, 1932) in the Papilionoideae.

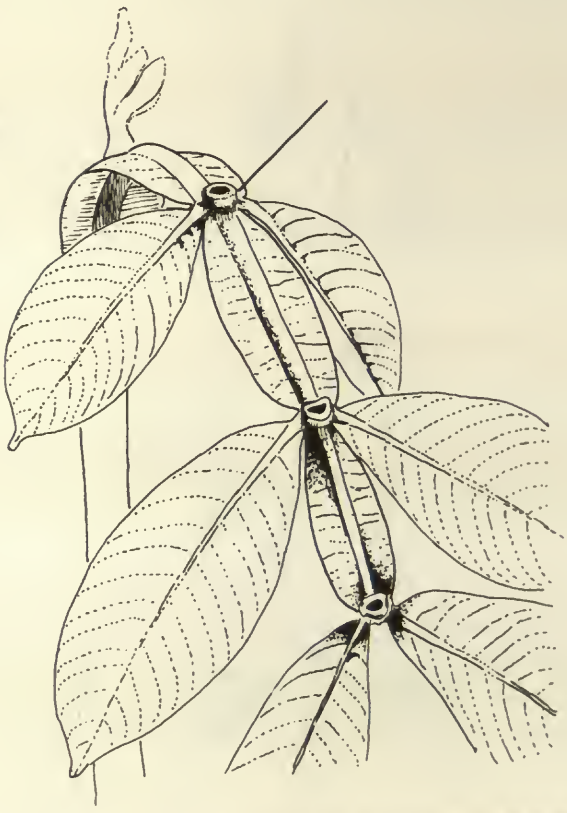

FIGURE 2. Petiolar/jugal nectaries of *Inga* sp. (Mimosoideae: Ingeae). Drawn from a photograph of a living plant, Finca La Selva, Costa Rica.

The situation is somewhat confused in the caesalpinioid tribes Detarieae and Amherstieae. Leaves of members of these groups often bear glands, and these have sometimes been considered nectaries (e.g., Bhattacharyya & Maheshwari, 1971b; Schnell et al., 1963; Cowan & Polhill, 1981). However, in only one case, a myrmecophyte (Detarieae: *Leonardoxa africana:* Elias, 1980; McKey, 1984), is it certain that the glands concerned are nectaries (see Fig. 6). These glands secrete a glucose- and fructose-rich nectar (McKey & Hossaert, unpublished). I could find no other record of even proximate analysis of composition of the secretion of leaf-surface and leaf-margin glands in the Detarieae and Amherstieae. A second myrmecophyte in this group, *Humboldtia laurifolia* (Amherstieae), also possesses ant-attractant glands that Bower (1887) regarded as nectaries. This is likely, but as Czapek (1909) pointed out, it has not been demonstrated. Cowan & Polhill (1981) mentioned "a wide variety of extrafloral nectaries (in the Detarieae and Amherstieae), most often a sticky patch overlying the petiolule insertion, but also a variety of specialized, and often generically characteristic, glands underneath or around the margins." These authors did not mention observations of ants at such glands. These glands need to be studied more closely, their secretions characterized, and the behavior of ants toward them observed in the field. At present there is little evidence for regarding them as nectaries. I suspect that some of them will turn out to be. Herbarium studies (McKey, unpublished) have shown that nonmyrmecophytic congeners of *Leonardoxa africana* possess small and inconspicuous glands similar in structure and location to the nectaries of this myrmecophyte. Similar glands occur on leaflet blades of *Afzelia, Plagiosiphon,* and other genera of

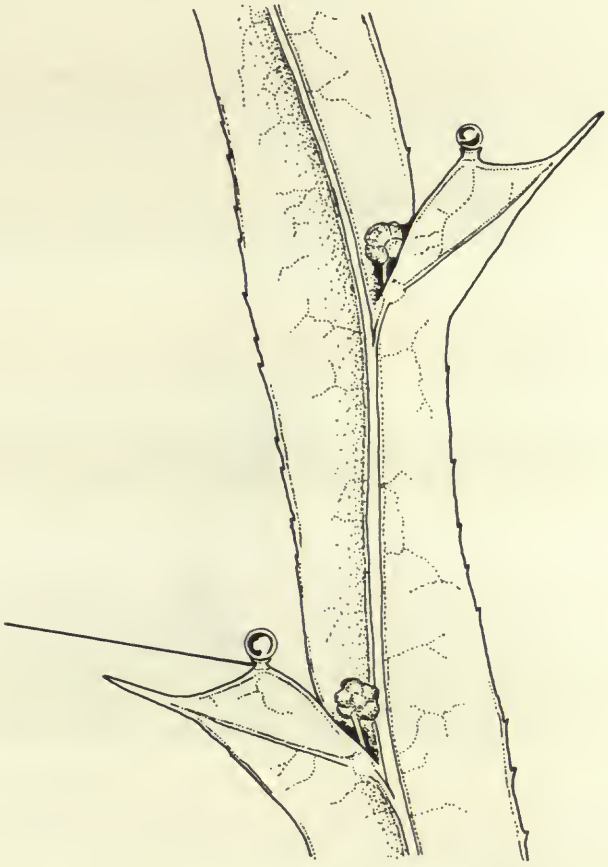

FIGURE 3. Extrafloral nectaries (arrow) of a phyllodineous *Acacia, A. alata* (Mimosoideae: Acacieae), each with a drop of nectar. Drawn from a photograph taken by Alexander Hansen.

Detarieae, and I have observed ants visiting foliar glands of *Plagiosiphon* in Sierra Leone, West Africa (McKey, unpublished). Nonmyrmecophytic species of *Humboldtia* also possess structures similar to the putative nectaries of *H. laurifolia* (McKey, unpublished).

Although extrafloral nectaries certainly occur in more Detarieae and Amherstieae than the literature indicates, in my experience foraging ants are encountered less frequently on these plants than on Mimosoideae. In those Detarieae and Amherstieae that do possess nectaries, they are smaller and secrete less nectar than the large petiolar/jugal nectaries of mimosoids. Table 2 is my attempt at a comprehensive survey of extrafloral nectaries present in legume genera, based on critical assessment of the literature, limited herbarium studies, and my own field observations. Though I cannot be sure, I believe that very few non-nectariferous glands appear in this table. Records were included that met one or more of the following criteria: 1) nectar secretion has been documented; 2) ant visitation has been noted; 3) homology is apparent with nectary glands in related genera. The last criterion was important in constructing the list for the Mimosoideae. In genera that have been studied, the glands on the leaf rachis are nectary glands, justifying the assumption that they will turn out to be nectaries in the other genera as well.

On the other hand, there are probably many additions to be made to the table. Further study may

FIGURE 4. Stipular nectaries of *Vicia* sp. (Papilionoideae: Vicieae). Drawn from a photograph of a living plant, near Basel, Switzerland.

show that some of the records I have excluded should have been included. New discoveries of inconspicuous extrafloral nectaries in legumes will also be made as more morphologists observe living plants in the field.

Patterns in the Taxonomic Distribution of Extrafloral Nectaries in the Leguminosae

Several patterns are evident from the information presented in Table 2. First, extrafloral nectaries apparently occur more frequently among the Mimosoideae than in the other two subfamilies. They occur in at least 70% of all mimosoid genera and are documented in all tribes except the monotypic Mimozygantheae. Furthermore, nectaries of similar structure occur in all four nectary-possessing tribes. Extrafloral nectaries of most mimosoids are slit- or cup-shaped petiolar or jugal glands (see Fig. 2). In the tribe Mimoseae, such nectaries have been documented in 24 of the 37 genera and appear to be absent in the other 13 (Lewis & Elias, 1981). In the Ingeae, published reports and drawings document the occurrence of such glands in at least 17 of the 20 genera; examination of herbarium specimens or living plants would probably push the figure to 100%. Most *Acacia* possess

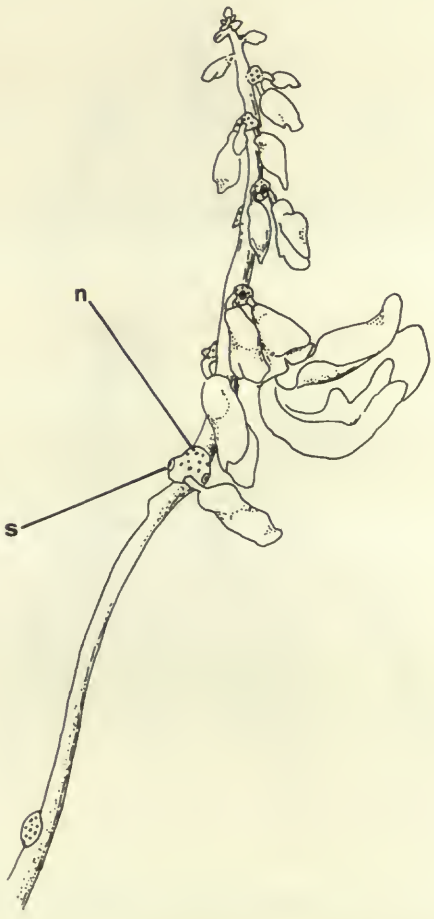

FIGURE 5. Extrafloral nectaries (n) of *Canavalia gladiata* (Papilionoideae: Phaseoleae). Also shown are scars (s) of fallen flowers. Redrawn from Nieuwenhuis von Uexkull-Gueldenbandt (1907).

extrafloral nectaries (Table 3), either on the rachis or on phyllodes, structures of petiolar origin (see Fig. 3). Three mimosoid genera possess extrafloral nectaries occurring on the shoot axis. Of these, *Pentaclethra* and *Entada* lack petiolar/jugal nectaries, while some species of *Pithecellobium* possess nectaries in both locations (Nieuwenhuis, 1907). As in many other plant groups, the extrafloral nectaries of Mimosoideae are most active when the leaves are young (e.g., Buckley, 1983; Koptur, 1984).

In the other two subfamilies, extrafloral nectaries occur in lower proportions of tribes and of genera within each tribe. In the Caesalpinioideae, nectaries appear to be virtually absent from tribe Caesalpinieae (including the basal groups Ceratoniinae, *Gleditsia* group, and Cercidinae); the only published reports in this tribe are questionable.

In some species of *Sclerolobium* (~ *Tachigali*), ants are frequently found on the foliate stipules (R. Foster, pers. comm.), but it is apparently not known whether these are glandular.

The tribe Cassieae contains the only group of Caesalpinioideae in which the widespread occurrence of extrafloral nectaries is well-documented. These are the genera *Senna* and *Chamaecrista*,

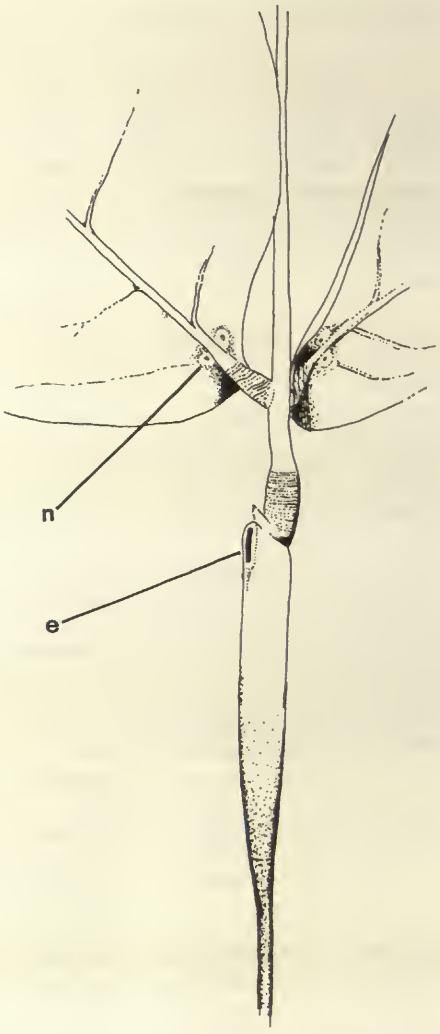

FIGURE 6. *Leonardoxa africana* (Caesalpinioideae: Detarieae), showing foliar nectaries (n) and entrance slit (e) made by *Petalomyrmex phylax* in the internode apex. Drawn from a photograph of a living plant, Douala-Edea Reserve, Cameroon.

formerly sections of *Cassia*. The genus *Cassia* as defined by Irwin & Barneby (1981) (= *Cassia* subg. *Cassia*) lacks extrafloral nectaries (Bhattacharyya & Maheshwari, 1971b; Irwin & Barneby, 1981). The petiolar and/or jugal nectaries of *Senna* and *Chamaecrista* are superficially similar to those of mimosoids. In addition, these plants sometimes bear nectaries on inflorescence axes. At least one species, *Senna alata*, bears nectaries on stipules, but not elsewhere (Zimmermann, 1932).

As noted above, information on nectary occurrence in the Detarieae and the Amherstieae is incomplete, but so far foliar nectaries have been documented in only one genus of each tribe, *Leonardoxa* and *Humboldtia*, respectively. Extrafloral nectaries appear to occur on structures other than leaves in at least two Amherstieae. Stipules of *H. laurifolia* possess glands similar to those

TABLE 2. Distribution of extrafloral nectaries in the Leguminosae. Genera according to treatments in Polhill & Raven (1981).

Subfamily	Tribe	Total number of genera	Number of genera with nectaries	Identity	Location on plant	References
CAESALPINIOIDEAE						
	Caesalpinieae	47	2	*Delonix*	*D. regia:* below first leaflet pair	Zimmermann, 1932
				Caesalpinia	*C. gilliesii:* leaflet margins	Rippa, 1903
	Cassieae	20	2	*Senna*	*S. alata:* stipules; many species: on rachis and/or raceme axes	Zimmermann, 1932
				Chamaecrista	many species: on rachis and/or raceme axes	Irwin & Barneby, 1981; Schnell et al.; 1963; Bhattacharyya & Maheshwari, 1971b
	Cercideae	5	1?	*Bauhinia*	?	O'Dowd, 1982
	Detarieae	55	1	*Leonardoxa*	leaflet underside at base	Elias, 1980; McKey, 1984, unpublished
	Amherstieae	25	2–4	*Brownea*	*B. grandiceps:* bud scales ?	Buesgen, 1903
				Humboldtia	*H. laurifolia:* leaflets and stipules	Bower, 1887
				(Gilbertiodendron, Pellegriniodendron)	on leaflet margins	Leonard, 1957; Schnell et al., 1963
MIMOSOIDEAE						
	Parkieae	2	2	*Parkia*	leaf rachis	Schnell et al., 1963
				Pentaclethra	*P. macroloba:* shoot axis	G. Hartshorn, pers. comm.
	Mimoseae	37	25	24 genera: leaf rachis (petiolar/jugal nectaries on leaf rachis absent in *Dinizia, Aubrevillea, Fillaeopsis, Piptadeniastrum, Adenanthera, Tetrapleura, Amblygonocarpus, Pseudoprosopis, Elephantorrhiza, Entada, Mimosa, Schrankia, Schrankiastrum*)		Lewis & Elias, 1981

TABLE 2. Continued.

Subfamily	Tribe	Total number of genera	Number of genera with nectaries	Identity	Location on plant	References
				Entada	on shoot axis below stipules	Zimmermann, 1932; Schnell et al., 1963
	Acacieae	2	1	*Acacia*	on rachis	Zimmermann, 1932; Bhattacharyya & Maheshwari, 1973; Janzen, 1974
					on phyllodes	Zimmermann, 1932; Majer, 1979; Buckley, 1983
					nectaries absent in single species of 2 sects. of subg. Phyllodineae and in sect. Filicinae of subg. Bipinnatae	Boughton, 1981, 1985
	Ingeae	20	at least 17, probably 20		on rachis	Zimmermann, 1932; Schnell et al., 1963; Elias, 1970; Bhattacharyya & Maheshwari, 1973; Koptur, 1984, 1985
PAPILIONOIDEAE						
	Robinieae	21	2	*Sesbania*	underside of midrib of basal leaflets	McKey & Hossaert, unpublished; R. Monteiro, pers. comm.
				Glottidium	underside of midrib of basal leaflets	McKey & Hossaert, unpublished
Phaseoleae subtribe Erythrininae		9	1	*Erythrina*	stipules, stipels, calyx	Zimmermann, 1932; Feinsinger et al., 1976; Sherbrooke & Sheerens, 1979
Diocleinae		13	1	*Canavalia*	in leaf axils and in inflorescence	Nieuwenhuis, 1907; Zimmermann, 1932

TABLE 2. Continued.

Subfamily	Tribe	Total number of genera	Number of genera with nectaries	Identity	Location on plant	References
Phaseolae subtribe						
Glycininae		16	1?	*Glycine*	base of leaflets	Zimmermann, 1932
Phaseolinae		23	5	*Physostigma*	in inflorescence	Zimmermann, 1932
				Vigna	in inflorescence	Zimmermann, 1932; Ojehomon, 1968
					in inflorescence, on stipels	Kuo & Pate, 1985; Pate et al., 1985
				Lablab	stipules, stipels	Zimmermann, 1932
				Dolichos	stipules, stipels and in inflorescence	Zimmermann, 1932
				Phaseolus	stipules, stipels and in inflorescnece	Zimmermann, 1932
	Loteae	4	1	*Lotus*	stipules	Polhill, 1981
	Coronilleae	6	1	*Hippocrepis*	stipules	Dominguez, 1976
	Vicieae	5	1	*Vicia*	stipules	Figier, 1971, 1972; Koptur, 1979, 1986;
					calyx	Kupicha, 1976
	Crotalarieae	16	1	*Crotalaria*	stumps of aborted bracts and flowers	Noack, 1903; Zimmermann, 1932; J. Anderson, pers. comm.

present on the leaves (Bower, 1887). Buesgen (1903) observed that leaf buds of *Brownea grandiceps* begin to secrete nectar just before opening, attracting numerous ants. From his meager description, it seems that nectaries may be present on the bud scales.

In contrast to the Mimosoideae, in which the majority of genera possess petiolar/jugal nectaries, there is no single widespread structural and developmental "plan" for nectaries of caesalpinioids. The nectaries (and putative nectaries) of those few caesalpinioids reported to possess them are a heterogeneous collection of structures occurring on leaves, stipules, and bud scales. Some are most active on buds and young leaves, but others, like those of *Leonardoxa africana*, are active primarily on mature leaves (McKey, 1984).

As shown in Table 2, a very small proportion of papilionoid genera have been reliably reported to possess extrafloral nectaries. Nectaries are also extremely unequally distributed among the various parts of the subfamily. There are no reliable records of nectaries in 26 of the 32 tribes treated in Polhill & Raven (1981). In four others, nectaries have been documented in a single genus. Only in one tribe, the Phaseoleae, do extrafloral nectaries occur in numerous genera. Within this tribe, nectaries occur in some subtribes but not in others, and are widespread in only two groups: the genus *Erythrina* and the subtribe Phaseolinae. Although new records of extrafloral nectaries doubtless

await discovery, the relative paucity of nectaries in the subfamily and their concentration in Phaseoleae appear to be robust patterns.

In the Sesbanieae, nectaries occur in *Sesbania* and in the closely allied monotypic genus *Glottidium*, in which we have recently found formless glands on the underside of the midrib of basal leaflets of pinnately compound leaves in seedlings of three species of the southeastern U.S. (McKey & Hossaert, unpublished). *Sesbania punicea* also possesses such nectaries (R. Monteiro, pers. comm.).

Stipular nectaries appear to occur in one genus each of three temperate herbaceous tribes, Loteae *(Lotus)*, Coronilleae *(Hippocrepis)*, and Vicieae (*Vicia;* Fig. 4). Of these, only in *Vicia*, in which nectaries occur only in one of the two subgenera (Kupicha, 1976), have nectaries been subject to morphological (Figier, 1971) and ecological (Koptur, 1979, 1986) studies. In some species of *Vicia* nectaries occur on the calyx lobes as well as on the stipules (Bisby & Maxted, pers. comm.). Similarly, some species of *Hippocrepis* possess nectaries on the bracts in addition to stipular nectaries (Dominguez, 1976).

In the Crotalarieae, a very different kind of extrafloral nectary was described by Noack (1903) for two species of *Crotalaria* in Brazil. Bracts of *C. anagyroides* fall from inflorescences once the flowers are open; the scars from the fallen bracts are glandular, secreting nectar and attracting ants, which, according to Noack, protect the inflorescences. In a second species *(C. striata?)* the bracts and some of the flowers are shed, and the stumps of the pedicels secrete nectar. In *C. intermedia* stumps of pedicels of shed flowers secrete nectar and ants are attracted (J. Anderson, Univ. of Florida, pers. comm.). Abscission of flowers (at various stages) or parts of inflorescences is quite common in the Papilionoideae, and this simple type of nectary might occur in other genera.

As noted above, only in the Phaseoleae do extrafloral nectaries occur in numerous genera. Even here, nectaries have been documented in only three of the eight subtribes (plus a questionable record for *Glycine* in the Glycininae). In *Erythrina* (subtribe Erythrininae), nectaries are widespread on the stipules and stipels and on the calyx. Formless nectaries are apparently common on the stipules and stipels of Phaseolinae; Zimmermann (1932) listed only three genera, but noted that such nectaries are present on other Phaseolinae (e.g., *Vigna:* Kuo & Pate, 1985; Pate et al., 1985). Observations of living plants are necessary to clarify patterns of occurrence of these inconspicuous nectaries. Kuo & Pate (1985) and Pate et al. (1985) described the structure and function of stipellar nectaries in *Vigna* and presented comparative information on other genera.

Several genera of Phaseoleae with nodose inflorescences possess an intriguing type of nectary that is functionally an extrafloral nectary but whose developmental roots may lie in floral structures. These nectaries occur in groups on the swollen mounds of tissue ("cushions"; reduced side-branches) on the axis of the pseudoraceme (Fig. 5). Anatomical (Zimmerman, 1932) and developmental (Ojehomon, 1968) studies in *Vigna* indicated that the nectaries may be derived from aborted flower buds. According to Ojehomon (1968), the flower buds on the cushion abort and drop, leaving an exposed scar which becomes tumescent and glandlike, secreting nectar and attracting ants, which might function in protection of the remaining flowers of the inflorescence. They are thus functionally similar to the differently produced extrafloral nectaries of the *Crotalaria* species studied by Noack (1903). The detailed studies of Kuo & Pate (1985) and Pate et al. (1985) called into question Ojehomon's (1968) interpretation of their ontogenetic derivation. See Tucker, this volume, for details on floral ontogeny.

The study by Pate et al. (1985) underlined an interesting functional feature of the inflorescence-stalk nectaries of *Vigna*. In contrast to most described nectaries, these extrude some intact cells and cellular debris, resulting in a secretion rich in amino acids relative to other floral and extrafloral nectars (Pate et al., 1985).

Phylogenetic Aspects of Nectary Evolution

Information on the distribution of extrafloral nectaries in the Leguminosae and on their structure (to distinguish homology from analogy), interpreted in the context of current ideas on the phylogeny

of the family, yields a tentative view of the evolutionary radiation of extrafloral nectaries and of facultative interactions with ants in the family.

According to Polhill et al. (1981), "The most archaic genera of Leguminosae seem to be the diverse extratropical woody Caesalpinioideae." If their views are correct, then the earliest legumes probably lacked extrafloral nectaries, because none of the genera referred to (*Gleditsia, Gymnocladus, Ceratonia, Zenia, Cercis*) appear to possess them, and because the plant families considered by these authors to be most closely related to legumes—Connaraceae and Sapindaceae—also lack nectaries, according to surveys by Delpino (1886–1889), Nieuwenhuis (1907), and Zimmermann (1932). The stock from which the Leguminosae arose, according to this view, thus probably lacked extrafloral nectaries. Several genera in another family sometimes considered closely related to legumes, the Chrysobalanaceae (Dickison, 1981), do possess extrafloral nectaries, as do some of their close relatives, the Rosaceae (Zimmermann, 1932).

Polhill et al. (1981) also proposed that the main radiation of the family may have stemmed from plants similar to the pantropical *Dimorphandra* group and the South American *Sclerolobium* group. These groups seem to lack extrafloral nectaries as well. Thus it seems possible that opportunistic interactions with ants mediated by extrafloral nectaries may not have been common in the early stages of legume radiation. The major uncertainty here concerns the *Sclerolobium* group. Two genera include myrmecophytes (see part IV), and the nature of the facultative ant-plant mutalisms from which these symbioses arose is unclear.

It does seem clear that petiolar/jugal nectaries became established fairly early in the mimosoid radiation. Exactly how early is an open question. Polhill et al. (1981) considered Mimoseae to be an older group than Acacieae and Ingeae, and it is interesting that 13 genera of this tribe lack this kind of nectary (Lewis & Elias, 1981). Is lack of petiolar/jugal nectaries a primitive trait in contemporary members of the tribe? The most generalized genera of the Mimoseae, *Dinizia, Aubrevillea,* and *Fillaeopsis,* all lack such nectaries (Lewis & Elias, 1981). The situation is complicated because secondary loss almost certainly occurs. This might account for the absence of nectaries in the shrubby or herbaceous genera such as *Mimosa, Schrankia,* and *Schrankiastrum.*

Except for the monotypic genus *Faidherbia,* petiolar/jugal nectaries seem to be a general feature of Acacieae and Ingeae.

The only other type of extrafloral nectary reported in mimosoids are stem-axis nectaries, present on *Pentaclethra, Entada,* and *Pithecellobium,* which belong to three different tribes. These appear to represent independent evolutionary events.

In the Caesalpinioideae, as noted before, nectaries seem absent in basal groups. The petiolar/jugal glands typical of two closely related genera of Cassieae appear to be independently evolved from those of mimosoids. In Detarieae and Amherstieae, where most uncertainty exists in patterns of nectary occurrence, those that have been documented are very different structures from those of other legumes (Elias, 1980).

In the Papilionoideae, extrafloral nectaries are again absent from basal tribes and, apart from the records for two species of *Crotalaria,* appear to have evolved in only two advanced tropical tribes (Sesbanieae and Phaseoleae) and in three temperate tribes closely related to one another (Loteae, Coronilleae, and Vicieae). One genus of each tribe possesses stipular nectaries. No morphological data exist to determine whether these represent independent evolutions. In *Vicia,* only plants of subg. *Vicia* possess nectaries. If Kupicha's (1976) opinion is correct that this subgenus is derived relative to subg. *Vicilla,* then nectaries would seem to have been independently evolved in this genus.

It is obvious, even from the preceding tentative analysis, that extrafloral nectaries have evolved independently many times in the Leguminosae. A very conservative estimate is that they have evolved at least 12 times: four times in the Mimosoideae (petiolar/jugal glands, and stem-axis glands of three widely divergent genera); at least three times, and probably more, in the Caesalpinioideae (Cassieae, *Leonardoxa* and *Humboldtia*); and at least five times in the Papilionoideae (Phaseoleae, *Sesbania-Glottidium,* Loteae-Coronilleae, *Vicia,* and *Crotalaria*). As knowledge

progresses, these estimates will probably be revised upward. *Erythrina*, as Lackey (1981) pointed out, is far removed from other Phaseoleae, so that its nectaries might have had an independent origin. Furthermore, as Table 2 shows, some legumes possess nectaries of different structure on different organs, so that nectaries may have evolved more than once in a single group.

EVOLUTIONARY ECOLOGY OF EXTRAFLORAL NECTARIES IN THE LEGUMINOSAE

The large number of independent evolutions of extrafloral nectaries in legumes, the great variety of morphological substrates that have given rise to nectaries in this and other plant families, and the relatively simple structural and physiological modification that can lead to extrafloral nectar production, suggest that extrafloral nectaries might be relatively "easy" structures to evolve, in this family as in others (cf. Bentley, 1977). Secondary loss also seems common (Bentley, 1977). The evolution of nectaries is thus probably less subject to historical constraints than that of many more complex ecological adaptations, and patterns of nectary presence and absence among legume taxa are likely to reflect adaptive differences rather than simply phylogenetic inertia. In this section, I present hypotheses to explain why some legume groups possess nectaries and others do not. This requires consideration of the other anti-herbivore defenses of legumes. I also present hypotheses to account for patterns in the intraplant distribution of nectaries on leguminous plants.

Role of Extrafloral Nectaries in the Anti-herbivore Defense System of the Plant

The defense system of the plant comprises several kinds of defense mechanisms, the most important being chemical and mechanical defenses, phenological defenses, and biotic defense, such as that provided by ants. Patterns of distribution suggest that the various kinds of chemical and mechanical defenses present in a single plant perform complementary functions, each being most suitable for defense of a particular subset of plant parts (McKey, 1979). Similarly, plants differing in life history and in leaf lifespan present adaptive differences in their chemical-defense profile (McKey, 1979; Waterman & McKey, 1989; Coley et al., 1985). Numerous studies have examined the relationships between chemical/mechanical defenses and phenological defenses, the escape of plants or plant parts from herbivores in time (Rhoades & Cates, 1976; Feeny, 1976; McKey, 1979). However, very few studies of interactions of ants and extrafloral-nectary-bearing plants have explicitly addressed the relationships that may exist between defense by ants and the other defenses of the plant (McKey, 1988). An examination of these relationships is central to the hypotheses presented here about distribution of extrafloral nectaries in leguminous plants. I will first discuss the role of biotic defenses in the protection of leaves.

Nectaries associated with vegetative structures are usually most active when the leaves are very young. This is true in many legumes (Nieuwenhuis, 1907; Koptur, 1984; Barton, 1986) as well as in many other nectary-bearing plants (Nieuwenhuis, 1907; Zimmermann, 1932; Bentley, 1977). I attribute this to the lack of effective chemical/mechanical defenses in most young leaves. Lignin renders leaves tough, conferring mechanical defense, and encrusts cellulose, making it indigestible. Expanding cells cannot yet be lignified. Tannin is deposited in vacuoles, and vacuolar space is limited in young expanding cells. A variety of low-molecular-weight secondary compounds are present in young leaves (McKey, 1974, 1979), but these are effective only against some herbivores. The effectiveness of chemical defenses in young leaves is further limited by the high nutrient content of their tissues, which means that a higher cost of detoxification (or other form of neutralization) can be supported by herbivores.

Two other factors may restrict nectar secretion and distribution of ant protection to young leaves in most plants with extrafloral nectaries. First, compared to tannins and lignin, defense by ants imposes a high maintenance cost, via nectar production, and may be favored only where tannins and

lignin are difficult to employ (McKey, 1984). Second, because young leaves have a greater future contribution to plant fitness than do old leaves, plants may be favored that protect young leaves well, whether by biotic or other defenses (McKey, 1979).

Where they occur among the legumes, nectaries on vegetative structures appear to function particularly in the protection of young leaves. Why do some legumes have them and others do not? In the absence of effective chemical/mechanical protection, plants may provide young leaves with biotic defenses, e.g., ants attracted to extrafloral nectaries, or phenological defenses, by modifying the temporal pattern of young leaf protection. What are the relationships between these two forms of young leaf protection? Synchronous production of large amounts of young leaves ("leaf outpourings" sensu Funke [1929]) can lead to escape of young leaves in time (Lieberman & Lieberman, 1984; Aide, 1988), analogous to satiation of seed predators (Janzen, 1971). This pattern of leaf production may conflict directly with effective protection by ants. Pulses of production of large amounts of young leaves, separated by long intervals, may result in satiation of ants, especially if nectaries are inactive in mature leaves, which is common in many species. Opportunistically foraging ants would have to be recruited with each new episode of leaf production, and the numbers are likely to be small in relation to the surface area of young leaves present. On the other hand, relatively continuous production of young leaves would result in no temporal escape from herbivores but would highly favor ant protection of young leaves if nectaries or other attractants are present. A resource that is predictably present is likely to consistently attract foraging ants, which could effectively patrol the small surface area of young leaves present at any one time. I predict that extrafloral nectaries active on young leaves will be more common on plants with relatively continuous young leaf production than on plants with highly pulsed production of young leaves.

One life form stands out by its relatively continuous production of young leaves: vines (Putz & Windsor, 1987). Vines are adapted to exploit light-rich environments. Success in the intense competition for light requires avoiding being shaded out by other plants. To the degree that other resources (e.g., water) are always available, competition in crowded and light-rich sites should engender a pattern of continuous growth. On Barro Colorado Island, individuals of vine species produced young leaves more continuously than did trees (Putz & Windsor, 1987).

Most large taxa of tropical vines are characterized by extrafloral nectaries active on young leaves and/or associated organs. Such nectaries appear to be widespread in tropical vines of the families Bignoniaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Malpighiaceae, and Passifloraceae (Zimmermann, 1932). Bentley (1981) found extrafloral nectaries on a greater proportion of vine species than species of other life forms in habitats surveyed in Costa Rica. She postulated that ants are recruited in larger numbers to nectaries on vines than on other plants because vine stems contact many other plants, each a potential source of ants. I believe that relatively continuous young leaf production is another reason for the high frequency of nectaries among vines. Among the legumes, the subtribe Phaseolinae of tribe Phaseoleae are tropical vines par excellence; stipular nectaries are frequent in this group. Vines in other legume taxa (e.g., *Entada, Acacia*) also possess nectaries. In another genus containing many tropical vines, *Bauhinia*, nectaries are questionably present, and other ant-attractant structures, pearl bodies, are documented (O'Dowd, 1982).

I suspect that contrasting patterns in the phenology of young leaf production may account for other patterns in extrafloral nectaries among legumes. Evaluation of this idea is hampered by a dearth of published information on patterns of young leaf production. Reproductive phenology of tropical trees has been rather extensively studied because interesting questions have arisen. Vegetative phenology, in contrast, has been poorly studied because these questions are lacking. The spirit of the following section is to offer tentative generalizations that will stimulate such studies.

Tropical tree representatives of the three legume subfamilies present a suite of ecological differences that lead to differences in phenology of young leaf production, and I argue, to differences in the frequency of extrafloral nectaries. Many caesalpinioids, particularly the Detarieae and the Amherstieae, can be classified using Grime's (1979) terminology as "stress-tolerators," adapted to

nutrient-poor or otherwise unfavorable sites. Relative to caesalpinioids, most tropical mimosoid and papilionoid trees are "competitors," plants with genetic characteristics that permit a high rate of acquisition of resources in productive, crowded vegetation, including the ability to make rapid adjustments in the distribution of individual leaves. This implies high rates of leaf turnover. In general, this is accomplished differently by mimosoid and papilionoid trees. Papilionoid trees tend to shed leaves synchronously within the tree and produce a large new crop of young leaves. This may be related to the low content of fungistatic and bacteriostatic tannins and lignin in leaves of this subfamily (Polhill et al., 1981): young leaves are safe only when old, often infected leaves have been shed. The pulsed production of crops of young leaves may account for the low frequency of foliar nectaries. Mimosoid trees, on the other hand, tend to shed and produce leaves relatively continuously; tannins and lignin in mature leaves (Polhill et al., 1981) may prevent pathogen buildup and transfer to young leaves. Whatever the reason, the relatively continuous leaf production typical of *Inga* species (Koptur, 1984) and other Mimosoideae may greatly favor young leaf protection by ants over alternative strategies of defense.

According to this view, ants attracted to extrafloral nectaries allow plants in crowded, productive environments to produce young leaves continuously (resulting in greater success in vegetative competition) without having these highly predictable and vulnerable tissues completely destroyed by herbivores. This may explain the high frequency of nectaries among vines, plants of secondary growth (Bentley, 1977), and fast-growing canopy trees.

Tropical caesalpinioid trees provide a striking contrast to such plants. This group, in particular the Detarieae and Amherstieae, tend to inhabit low-nutrient or otherwise unfavorable sites. As expected for stress-tolerators (Grime, 1979), they have long-lived leaves rich in tannin and lignin (McKey, 1984). They also have a vegetative phenology characterized by intermittent production of young leaves. Growth in stress-tolerators usually occurs intermittently (Grime, 1979), leaves being produced during periods relatively favorable for growth. Once a pattern of intermittent leaf production is established, selection can act to intensify it by favoring individuals that most effectively satiate young leaf herbivores. I believe this has been an important selective factor in the evolution of the young leaf phenology of the Caesalpinioideae, a group that offers some of the most spectacular examples known of rapid maturation of large crops of synchronously produced young leaves. The phenomenon of "pouring out" of entire young branch segments, each bearing several young leaves, synchronously over the crown or a good portion of it, is well known for many genera in the Detarieae and Amherstieae: *Maniltoa, Brownea, Amherstia, Saraca, Humboldtia, Cynometra,* and others (Funke, 1929; Schimper, 1935; Mueller, 1988), including *Daniellia, Isoberlinia* (Richards, 1952), *Berlinia, Anthonotha, Gilbertiodendron,* and *Baikiaea* (pers. observ.). The following quote from Keeble (1895) attests to the speed at which young leaves of these trees expand: "An unopened bud of *Brownea grandiceps* was ten inches long at six p.m. The next morning at seven o'clock the bud had opened and the petioles of the pinnate leaves which had issued from it were each a foot and a half in length. During the next two days they grew at the rate of little less than a foot a day, and had at the end of that time practically attained their full length." Such rapid leaf expansion, combined with pulsed production, is ideally suited for a tactic of escape from herbivores in time, but might render effective protection of young leaves by ants difficult.

As noted previously, it is not yet clear how many of these trees possess foliar nectaries. However, it seems clear that in those few with documented nectaries, the biology of the interaction is somewhat different than in nectary-bearing Mimosoideae. My own unpublished observations on *Leonardoxa africana* (Detarieae) and closely related congeners provide insight here. In contrast to populations in the Cameroon coastal plain (McKey, 1984), populations in noncoastal southern Cameroon and in Gabon are not myrmecophytes, but possess small foliar nectaries that attract a variety of opportunistically foraging ants. In contrast to the nectaries of mimosoids and most other plants, the nectaries of *Leonardoxa* are very active on mature leaves. A consequence is that nectar is continuously produced by these evergreen trees, although young leaves are produced in seasonal pulses. Near Mbalmayo, Cameroon, adult trees of *Leonardoxa* are occupied by the aggressive

myrmicine *Tetramorium aculeatum* (= *Macromischoides aculeatus*), which builds carton nests on leaf undersides. Thus ants are already present in large numbers on the tree when a pulse of young leaves is produced, and can be readily recruited to patrol these leaves.

A closer look must be given to trees like *Brownea grandiceps*. This species produces pulses of branch segments with several leaves that are rapidly expanded and matured (see above). From Buesgen's (1903) tantalizingly cursory remarks made in passing in a paper on growth of young *Brownea* leaves, it appears that the bud scales possess nectaries, which attract large numbers of ants upon bud opening. Where do the ants come from? I suspect that as in *Leonardoxa,* mature leaves may secrete small quantities of nectar so that foraging trails are established and workers can be recruited to young leaves when these are produced.

One group of caesalpinioids has many features in common with the mimosoid pattern: the genera *Senna* and *Chamaecrista*. In contrast to other Caesalpinioideae, these "weedy" caesalpinioids, like most mimosoids and papilionoids, fix nitrogen and are not characteristic of particularly nutrient-poor sites. Like Mimosoideae, they are competitors (Grime, 1979) that maintain their place in the sun by producing young leaves relatively continuously. They possess petiolar/jugal nectaries that appear to be most active on young leaves. It is perhaps significant that of the three genera constructed from the old genus *Cassia,* the one that lacks nectaries *(Cassia)* also does not fix nitrogen (Irwin & Barneby, 1981). I suspect this may reflect underlying ecological differences that affect competitive strategy, the phenology of leaf production, and in turn the adaptive value of nectaries.

Ant Defense of Reproductive Structures

In the Mimosoideae and in all caesalpinioids except the related genera *Senna* and *Chamaecrista,* and in some papilionoids *(Sesbania* and *Lotus),* extrafloral nectaries have been noted to occur only on vegetative structures of the plant. In *Senna* and *Chamaecrista* and in several papilionoid groups, however, extrafloral nectaries also occur on reproductive structures, including inflorescence axes *(Senna* and *Chamaecrista),* calyx (*Erythrina,* some *Vicia* species), bracts *(Hippocrepis),* cushions of nodose inflorescences (*Canavalia* and Phaseolinae), and scars of shed bracts and stumps of pedicels of aborted flowers *(Crotalaria).* With the exception of the last named genus, all of these possess extrafloral nectaries on vegetative structures as well. Thus there are two common patterns of intraplant distribution of extrafloral nectaries in legumes: vegetative structures only, and vegetative and reproductive structures. Most legume genera exhibiting the first pattern are trees; most genera in the second pattern are relatively short-lived shrubs, suffrutescent or herbaceous vines, and herbs. Similar contrasts in nectary distribution among vegetative and reproductive structures in long- and short-lived plants, respectively, are evident in other plant families (Zimmermann, 1932). As in the legumes, many families contain genera possessing nectaries on both classes of structures. Families for which nectaries are recorded only on vegetative structures (e.g., Anacardiaceae, Combretaceae, Ebenaceae) are mostly woody plants. Families with extrafloral nectaries occurring exclusively or almost so on reproductive structures are all herbaceous plants. In the Compositae, for example, extrafloral nectaries are known only on involucral leaves, with the exception of *Helianthus,* where they occur on the undersides of the uppermost leaves near the flowering part of the stem (Zimmerman, 1932). The Liliaceae present a similar pattern; only *Smilax* species, which are vines, possess extrafloral nectaries outside the inflorescence (Zimmermann, 1932).

This difference in the distribution of nectaries between short- and long-lived plants perhaps exemplifies a widespread difference in allocation of resources to vegetative and reproductive functions between plants differing in longevity (see Bloom et al., 1985, for review): short-lived plants invest more in reproduction—and in defense of reproductive structures by ants?—than do long-lived plants, which invest proportionally more in vegetative structures in order to reproduce later.

In some cases, nectaries on vegetative structures may also function in protection of reproductive parts. Ants attracted to the petiolar nectaries of *Chamaecrista fasciculata* increased plant reproduc-

tion primarily by removing a major predator of flowers, pods, and young leaves (Barton, 1986). The stipular nectaries of *Vicia* should be well placed to provide protection for the sessile axillary flowers of these species, as well as for the leaves. It is significant that in this genus stipular nectaries and very short inflorescences are always associated characters. *Vicia* subg. *Vicilla*, which possess multiflowered inflorescences with long peduncles, do not possess stipular nectaries. *Vicia* subg. *Vicia* are characterized by short, one-to-few-flowered inflorescences (sometimes sessile flowers), the flowers and developing fruits thus being in close proximity to the stipules. All members of this subgenus possess stipular nectaries (Kupicha, 1976). Stipular nectaries of other herbaceous papilionoids *(Lotus, Hippocrepis)* might also protect flowers or developing fruits, since they are likely to be most active at young, apical nodes near the inflorescences.

Extrafloral Nectaries on Multiple Organs of the Plant

In many plants belonging to a wide variety of families, extrafloral nectaries occur on two or more organs (Zimmerman, 1932). As noted above, the legumes offer many examples, all but one of which *(Pithecellobium)* concern the subfamilies Caesalpinioideae and Papilionoideae. In these two subfamilies, a little fewer than half of all nectary-bearing genera possess nectaries on two organs.

Why is this so? Polhill (in Polhill & Raven, 1981), noting the structural similarity of nectaries on different parts of papilionoids possessing them on both stipules and calyx or bracts, suggested that the same set of genetic information might be expressed in different organs. This would be an example of homeosis in plants (Sattler, 1988). This suggests a developmental/genetic mechanism for the evolution of nectaries in multiple sites, but does not explain why such a developmental pattern would be preserved by natural selection.

Two factors may combine to provide a selective advantage for plants that possess nectaries on two or more organs. First, as in plant chemical defenses (McKey, 1979), selection may favor the extension of an effective new defense to several parts of the plant. Furthermore, by attracting ants to the plant, nectaries on one part increase the chance that nectaries appearing elsewhere on the plant will be found and patrolled by ants and thus confer a selective advantage. In effect, by increasing overall levels of ant activity, nectaries on one part facilitate the evolution of nectaries on other parts of the plant. Second, if nectar-producing organs differ in temporal pattern of availability, nectar production by the plant is more continuous than if only one organ secretes nectar. The plant is a more constant and predictable food source for opportunistically foraging ants, and ants are more likely to maintain foraging trails to the plant. In effect, the greater constancy of nectar production by the plant as a whole enhances ant protection of each part. The importance of this mechanism was suggested by Sherbrooke & Scheerens (1979) for *Erythrina flabelliformis*. In this species of the southwestern U.S., nectaries on the calyces produce nectar throughout the winter when the plant is leafless, and nectaries on the stipules and stipels produce nectar throughout the summer when no flowers are present. This phenological pattern, expressed in tropical species as dry-season flowering and production of a crop of leaves at the start of the wet season, is general in the genus *Erythrina*. The system described for *E. flabelliformis*, in which an individual plant produces extrafloral nectar more or less continuously, requires that the flowering season be long and stipular nectaries be active throughout the lifespan of the leaf. The extended flowering season of *Erythrina* species, associated with pollination by traplining hummingbirds (Neill, pers. comm.), may have been an important preadaptation in the evolution of this system. It would be interesting to determine whether stipular nectaries of other species of the genus are also active for extended periods.

The hypotheses presented here to explain the patterns of extrafloral nectary occurrence in the Leguminosae emphasize the importance of predictability and constancy of nectar production for the recruitment of opportunistically foraging ants in numbers adequate to provide protection. At a finer level, a degree of unpredictability in nectar production may also enhance protection. If the plant consistently possesses some active nectaries but the location of individual active nectaries on the

plant varies unpredictably over time, ants would be expected to patrol greater areas of the plant's crown in their search for active nectaries, enhancing the effectiveness of protection (B. Bentley, pers. comm.).

The plant may also exert a degree of control over the effectiveness of ant patrolling by placement of nectaries at strategic locations and by timing their activity to coincide with vulnerable phases of development. In addition to the patterns already noted—maximum activity on young leaves in most species, placement of nectaries near flowers and developing fruits—another feature of nectary distribution may be important in enhancing effectiveness of ants. As in many other plant families (Zimmermann, 1932; Schnell et al., 1963; Jolivet, 1986), nectaries on vegetative structures of leguminous plants are usually located at or near the base of leaves or pinnae: on stipules, on the petiole (at the base of the rachis or at jugae), or at the base of leaflets. This pattern lacks an adequate explanation. Previous authors have invoked "leaf polarity" (Schnell et al., 1963; Jolivet, 1986), and believe that morphogenetic or physiological constraints (e.g., gradients in nutrient supply via phloem?) usually preclude the evolution of nectaries in apical parts of leaves or leaflets. I propose that the location of nectaries at the base of leaves, pinnae, or leaflets places the ant guard at the gate. By concentrating ant activity at the point where insect herbivores gain access to a leaf or leave it for another, the plant maximizes the protection gained through production of a limited quantity of nectar. Like all defenses, this one is not absolute. The blockade would presumably have less effect on insects that fly to a leaf to feed or oviposit than on walking insect herbivores.

IV. Legume Myrmecophytes

Like many other families with tropical woody representatives, the Leguminosae include a number of myrmecophytes, plants with more or less specialized structures (myrmecodomatia) that consistently host ant nests. Even though the world is not neatly divided into specialized symbioses vs. completely generalized opportunistic systems, interactions of these plants with ants are presumed to differ in several ways from those described above. First, because ants nest in the plant, levels of ant activity on domatia-bearing plants are higher and more constant in space and time than on plants with extrafloral nectaries or other ant attractants but lacking myrmecodomatia. Second, myrmecophytes are usually associated with a smaller number of ant species than are plants lacking myrmecodomatia. Third, the interaction is often obligate and plant species-specific for the ants and sometimes obligate for the plant as well.

Myrmecophytes probably differ in their degree of dependence on ants, and plant-ants probably differ in their host-plant specificity. Conversely, interactions among ants and some nonmyrmecophytes may approach some symbiotic interactions in their degree of specificity and interdependence. The ecology of arboreally foraging and nesting ants is very poorly investigated. The various plants in the habitat vary in quality as foraging and/or nesting substrates for different ant species, and various ant species have differing effects on plants.

Domatia-bearing plants presumably evolved from plants engaged in facultative interactions with ants. What selective advantages led to the evolution of ant-domatia? By providing nesting sites for ants, domatia lead to greater constancy of ant association with the plant than in systems where ants forage opportunistically on the plant and nest elsewhere. Greater constancy of ant presence leads to enhanced protection and presumably provides the selective environment for evolutionary interactions leading to obligate interdependence with specific ants (Schemske, 1983). However, many arboricolous ants construct nests on surfaces of living plants. *Crematogaster* constructs carton nests; *Oecophylla* nests in leaves bound by silk; *Macromischoides* builds felt nests on leaf undersides (Wilson, 1959; Leston, 1973; Carroll, 1979). Presence of domatia is not essential for constant association with nesting ants. Domatia, however, provide two important resources for arboricolous ants. First, they provide greater freedom from predation for resident ant colonies than enjoyed by ants nesting on exposed plant surfaces. Second, they provide convenient access to phloem and

protection from predation for ant-associated Homoptera. These symbionts are important in the evolution of close association with resident ants because they provide a protein-rich food supply complementary to carbohydrate-rich extrafloral nectar, permitting resident ants to obtain all their requirements in the crown of a single plant.

Myrmecophytes appear to have evolved at least seven times in the Leguminosae and occur in all three subfamilies. Four caesalpinioid genera (probably representing only three independent origins) contain myrmecophytic species. In the mimosoids, ant-plants have evolved twice in the genus *Acacia* and doubtfully in one or two other genera. At least two, and possibly up to eleven, widely separated papilionoid genera include ant-plants. Of the seven to 19 independent evolutionary origins of myrmecophytes in the legumes, three have culminated in modest radiations of several species of myrmecophytes; in the others, only one or two myrmecophytic species are known. Table 3 summarizes the information available on legumes reported to be myrmecophytic. Each of these will be discussed here.

I will first summarize what is known about these plants, especially the lesser-studied ones, in terms of life form and general natural history of the plant, the nature of domatia, identity of occupant ants, specificity of the ants, trophic basis of the interaction, and demonstrated or suspected benefits to the plant. I document the ecological diversity of legume ant-plants and show how features of habitat and associated features of plant strategies have led evolutionarily to important differences in features of the ant-plant interaction. Second, by comparing these plants with their nonmyrmecophytic relatives, I will examine hypotheses about how symbiotic, specific interactions may have evolved from the nonsymbiotic, generalized ant-plant interactions discussed in the previous section.

A SURVEY OF LEGUMINOUS ANT-PLANTS

In the Caesalpinioideae, myrmecophytes have evolved in all three major tropical land masses. *Tachigali* and *Sclerolobium*, two closely related South American genera of rainforest trees that in Dwyer's (1954, 1957) opinion are probably congeneric, each contain myrmecophytic species. Cladistic analysis of this group should show whether myrmecophytic traits are monophyletic. Ant domatia in these legumes are located in the swollen basal part of the petiole and rachis of the pinnately compound leaves (Fig. 7). Of the 22 species treated by Dwyer (1954) under *Tachigali*, herbarium specimens of 16 possessed such domatia. Dwyer noted that some material of these species lack domatia. It is not clear whether this reflects geographical or intrapopulational variation, or whether domatia are simply lacking in some leaves of individual trees.

The only species of *Tachigali* in which ant-plant interactions have been studied in the field is *T. paniculata*, studied in former British Guiana by Bailey (1923) and by Wheeler (1921a, b, 1942). Wheeler found adult trees of this species to be occupied by colonies of one of three obligate *Tachigali*-inhabiting ants, *Azteca foveiceps* (Dolichoderinae) or, more commonly, one of two *Pseudomyrmex* species, *P. damnosa* and *P. maligna*. Other species of *Pseudomyrmex* appear to be obligate inhabitants of *Tachigali* species elsewhere (Wheeler, 1942). The ants enter and excavate the swollen petioles. They tend mealy bugs *(Pseudococcus);* these feed on the nutritive parenchyma lining the inner walls of the petiolar cavities. The ants apparently eat the homopterans and not honeydew. These ants fiercely bite and sting intruders on their host plant, and Wheeler's (1921b) description of the system provides circumstantial evidence that the obligate *Tachigali*-inhabiting *Pseudomyrmex* protect the host from a variety of herbivores.

Juveniles of *Tachigali paniculata* growing in shaded understory are inhabited by a diversity of ants that are not specific to *Tachigali* and appear to provide no protection. They are also inhabited by two species of subsocial silvanid beetles (Wheeler, 1921b). All of these use the domatia, the nutritive parenchyma lining it, and the mealybugs, in the same way as the obligate *Tachigali*-

TABLE 3. Legume myrmecophytes, including some questionable records.[1]

Subfamily	Genus	Distribution	Number of species	Number with ant domatia	Nature of ant domatia	Trophic basis of interaction	Principal references
CAESALPINIOIDEAE							
	Tachigali	mostly Amazonian	24	16	fusiform swellings at petiole and base of rachis; no marked prostoma	homopterans *(Pseudococcus)* tended in domatia; nutritive parenchyma	Wheeler, 1921a, b; 1942; Bailey, 1923; Dwyer, 1954
	Sclerolobium	mostly Amazonian	35	6	as in *Tachigali*	?	Dwyer, 1954, 1957; Wheeler, 1942
				?	no petiolar swelling, but ants associated with foliate stipules	?	R. Foster, pers. comm.
	Leonardoxa	Guineo-Congolian	3	1	swollen internodes	extrafloral nectaries	Elias, 1980; McKey, 1984
	Humboldtia	S. India & Sri Lanka	6	1	swollen internodes	extrafloral nectaries ? homopterans ?	Bower, 1887; Escherich, 1911
MIMOSOIDEAE							
	Acacia	pantropical	ca. 1,200				
	neotropical:	Central America and N. South America		12	swollen stipular thorns	extrafloral nectaries, beltian bodies	Janzen, 1974 and refs. cited therein; Rickson, 1969, 1975; Seigler & Ebinger, 1987
	African:	E. African savannas		? (Hocking lists 15 species)	swollen stipular thorns	extrafloral nectaries, homopterans tended in thorns	Brown, 1960; Hocking, 1970; Ross, 1981
	Inga	tropical and subtropical America	ca. 350	1?	swollen twigs	extrafloral nectaries	Ducke, 1925a, b; Ducke & Black, 1953
	Pithecellobium	Central & South America	ca. 20	1?	swollen rachis *(P. incuriale)*	?	G. Lewis, pers. comm.

TABLE 3. Continued.

Subfamily	Genus	Distrubution	Number of species	Number with ant domatia	Nature of ant domatia	Trophic basis of interaction	Principal references
PAPILIONOIDEAE							
	Swartzia	neotropical, except for 2 African species	ca. 135	I?	hollow twigs and branches	?	Forel, 1904; Ule, 1905
	Ateleia	Mexico, W. Indies, Central & South America	16	1? (*A. pterocarpa* Sessé & Moc.)	hollow branchlets; 1–2 mm entrance holes, many on upper parts of inflorescence axes	?	Herbarium specimen *Neill 5397* (MO): C. Stirton, pers. comm.
	Ormosia	E.S. America, E. Asia to NE Australia	ca. 100	1? (*O. macrophylla*)	hollow stems		Rudd, 1965; J. Zarucchi, pers. comm.
	Alexa	trop. South America	7–9	1? (*A. cowanii*)	hollow twigs	?	C. Stirton, pers. comm.
	Andira	neotropical, with I sp. to Africa	ca. 20	1?	swollen inflorescence axes and twigs subtending inflorescences	?	Herbarium specimen *Begozo 184* (MO): C. Stirton, pers. comm.
	Platymiscium	W. Indies, Central & South America	ca. 20	2	hollow twigs	?	Wheeler, 1942
	Pterocarpus	pantropical	ca. 20	1	swollen inflorescence axes and twigs	?	Amshoff, 1939; Wheeler, 1942
	Lonchocarpus	tropical America and Africa	ca. 100	I?	petioles and rachis inflated, occupied by ants	?	A. Gentry, pers. comm.
	Whitfordiodendron	SE Asia, N. Australia to the Solomon Islands	ca. 15	2?	hollow stems	?	R. Geesink, pers. comm.
	Mastersia	Indo-Malaya	2	?	hollow stems	?	R. Geesink, pers. comm.
	Spatholobus	tropical Asia	15	I?	hollow stems	?	Ridder-Numan & Wiriadinata, 1985

[1]See also note added in proof.

FIGURE 7. Inflated petioles, with ant entrance holes, of *Tachigali cavipes* (Caesalpinioideae: Caesalpinieae). Drawn from a herbarium specimen (*Maguire & Wurdack 35,591*, Paris).

inhabiting ants do. Since these insects can exclude the obligate ants from plants they occupy (Wheeler, 1921b), they can be viewed as parasites on the mutualism rather than as commensals (cf. Janzen, 1975; McKey, 1984).

Though *Tachigali* figures in at least two lists of legumes bearing extrafloral nectaries (Bhattacharyya & Maheshwari, 1971b; Bentley, 1977), as stated earlier I have encountered no evidence to prove this. Bailey (1923) mentioned the absence of conspicuous extrafloral nectaries or food bodies on *T. paniculata*, and Wheeler's studies did not mention them.

Of the 35 species treated under *Sclerolobium* by Dwyer (1957), at least six possess swollen, hollow petioles inhabited by ants. What little is known about these appears in Table 3. Some species of *Sclerolobium* that lack petiolar domatia possess recurved foliate stipules, beneath which ants may nest (R. Foster & A. Gentry, pers. comm.). The recent publication by Benson (1985) presented information and discussion of *Tachigali* and *Sclerolobium*.

Leonardoxa, in the Detarieae, consists of three Central African species. Plants of coastal Cameroonian populations of one species, *L. africana*, are myrmecophytes (McKey, 1984). In these

plants (Fig. 6), the internodes of young twigs are swollen and filled with thick pith. The apex of the internode is most swollen, and the internode tapers from apex to base. The base is slender and not hollow. As in many other ant-plants, ants make entrance holes in a relatively unlignified part of the swollen stem, a feature known as the prostoma (e.g., in *Cecropia:* Janzen, 1973; *Triplaris:* Wheeler, 1942). In *L. africana,* each internode possesses a single prostoma at its apex, just below the next internode (or terminal bud) on the side opposite the point of leaf attachment. Ants excavate the pith and inhabit the hollow internode. The ants' principal food appears to be supplied by foliar nectaries, of which one–five are present at the base of each leaflet. These nectaries have been described by Elias (1980). Food bodies and other food-producing tissues appear to be absent. Plants require colonies of the ant *Petalomyrmex phylax* (Formicinae) for survival to reproductive maturity. These tiny ants, about 2 mm long, effectively protect the young leaves of the plant from destruction by chrysomelid beetles and microlepidopteran larvae (McKey, 1984). As in *Tachigali,* there is a persistent parasite of the mutualism. The ant *Cataulacus mckeyi* (Myrmicinae) occupies juvenile trees, does not protect them, and prevents reestablishment of the mutalist *Petalomyrmex* (McKey, 1984).

The *Leonardoxa/Petalomyrmex* system presents several differences from well-known ant-plants such as the neotropical ant-acacias, which seem to be evolutionary consequences of the light-poor understory habitat of this plant. Plants in such habitats have long-lived leaves, and this affects the costs and benefits of biotic defense of mature leaves by ants, relative to chemical/mechanical defense. In neotropical acacias, as in other ant-plants in light-rich habitats, leaves are short-lived, both young and mature leaves are patrolled by ants, and mature leaves often lack chemical defenses widespread in non-ant-acacias (Rehr et al., 1973; see also Seigler & Ebinger, 1987). In *Leonardoxa,* leaves are long-lived (3.5 years or more), ant patrolling is largely restricted to young leaves, and mature leaves are chemically and mechanically protected. I have argued (McKey, 1984) that in ant-plants with long-lived leaves, one-time investment in tannins and lignins—types of chemical defenses with low turnover and hence low maintenance costs—is cheaper than maintenance of ants over the long leaf lifespan. The plant maintains a smaller worker force that patrols only the vulnerable young leaves. In ant-plants with short-lived leaves, tannin and lignin present higher costs than ants because they are lost with leaves, and the high rate of leaf turnover means a high rate of loss (McKey, 1984).

Light- and energy-poor habitats might have selected for other features of the *Leonardoxa/Petalomyrmex* system, such as low colony biomass, finer subdivision of limited colony biomass among smaller workers, and even multiple-queen colonies (McKey, 1984).

Further work in progress has shown that *L. africana* consists of three distinct geographical varieties, whose taxonomic status will be formalized in an upcoming publication (McKey, in press). Those found in the Rumpi Hills area north of Mt. Cameroon are also myrmecophytes, but present some morphological differences from those on the coast, and are occupied by different ants. Populations of this species in noncoastal southern Cameroon and Gabon are nonmyrmecophytes, like the other two species in the genus, which occur in the Zaire basin.

Humboldtia laurifolia (Amherstieae), a little-known ant-plant of South India and Sri Lanka (Bower, 1887), is, like *L. africana,* a small tree of rainforest understory. The internodal swellings are superficially very similar to those of *Leonardoxa,* the swellings being most pronounced at the apex. As in *Leonardoxa,* a prostoma is also present near the internode apex, on the side of the axis opposite the insertion of the leaf. According to Bower's original description, only the upper internodes of each shoot are swollen. Glandular structures that are probably nectaries (Bower, 1887; but see Czapek, 1909) occur on the leaflets and stipules. Similar but smaller glands are found on leaves of nonmyrmecophytic *Humboldtia* species (McKey, unpublished). Bower noted *H. laurifolia* to be consistently occupied by "small black ants"; Escherich (1911) listed several ants he found in *Humboldtia* trees, and noted that one *Crematogaster* sp. tended coccids. His observations, however, like those of Czapek (1909) appear to have been carried out exclusively in botanical gardens. The interaction of *Humboldtia* with ants in natural habitats is completely undescribed. *Humboldtia*

FIGURE 8. *Pterocarpus amazonum* (Papilionoideae: Dalbergieae), showing inflated inflorescence axes and inflated hollow terminal twig section. Note ant entrance holes (arrows) in inflorescence axes, twig, and rachis of pinnately compound leaf. Drawn from a herbarium specimen (*Spruce 1533*, Kew).

appears to be convergent or parallel morphologically with *Leonardoxa*. Ecological studies of its interactions with ants would provide a test of hypotheses about the traits of understory ant-plant systems (McKey, 1984).

In the Mimosoideae, myrmecophytes have evolved at least twice in the genus *Acacia*, once in the northern Neotropics and once in East Africa. Both groups of myrmecophytic *Acacia* belong to the single subgenus *Acacia*, characterized by spinescent stipules (Ross, 1981), and in both it is the stipular thorns that have evolved into myrmecodomatia. In older literature, the South American species *A. caven* is also listed as a myrmecophyte, but as Janzen (1974) argued, this seems to be in error.

The Central American ant-acacias (12 species) and their interaction with obligate acacia-ants (*Pseudomyrmex* species) that subsist on the foliar nectar and beltian bodies produced by the plants are too well known for extensive comments in this survey to have any value. The monograph by

Janzen (1974) provides access to the literature, along with the references listed in Table 3. Of relevance here are Janzen's (1974) conclusions that the neotropical ant-acacias do not form a tight phyletic group, and that the ant-acacia life form spread among various species by introgression, producing a pattern of reticulate evolution.

The East African swollen-thorn acacias have been studied by Hocking (1970), who provided an exhaustive listing of older work on these plants. Hocking, who worked primarily on *A. drepanolobium,* listed 15 East African species with swollen thorns. The obligate ant inhabitants of *A. drepanolobium* are two species of *Crematogaster* (Myrmicinae) and the pseudomyrmecine *Tetraponera penzigi*. Like the American ant-acacias, the African species possess domatia that evolved from stipular thorns, and the occupant ants obtain much of their food from foliar nectaries. However, the two systems present marked differences, some of which have been summarized by Ross (1981). The African species lack beltian bodies and year-round leaf production and are not confined to the wettest areas of the seasonal African tropics. Extrafloral nectaries are not developed to the same extent. As Hocking (1970) documented, the obligate ants of the African acacias tend coccids in the hollow thorns. This source of food probably evens out the food supply to the ants in these habitats where foliar nectar production is highly seasonal. The Central American ant-acacias are found in the wettest parts of the neotropical range of this genus, and their relatively constant leaf production ensures a regular supply of foliar nectar and beltian bodies (Janzen, 1974).

The hot, dry sites where most African swollen-thorn acacias occur has had evolutionary consequences for other features of the system. Swollen thorns of *Acacia* species in such sites have white surfaces adapted to reflecting solar radiation and keeping the thorn interior cool. The black thorns of swollen-thorn acacias at higher elevations absorb solar radiation and keep the thorn interior warm (Hocking, 1970).

Ross (1981) concluded that, like the neotropical ant-acacias, the African swollen-thorn acacias do not consist of a group of closely related species. Whether hybridization of swollen-thorn acacias with nonmyrmecophytic species occurs—which would make plausible a mechanism leading to reticulate evolution like that postulated for the neotropical acacias by Janzen (1974)—is apparently unknown. Some taxa are intermediate in morphological traits related to ants and apparently in their degree of ant-dependence.

The only other mimosoid I have found mentioned in the literature as a possible myrmecophyte is an Amazonian species of the large genus *Inga, I. cinnamomea,* which appears in the list of Amazonian myrmecophytes included in Ducke & Black's (1953) paper on the phytogeography of the Amazon basin. According to Ducke (1925a, b), the twigs of this tree, which reaches 30 m in flood-prone forest along the banks of the Amazon and its tributaries, are hollow and frequently inhabited by *Pseudomyrmex* ants. Of the 12 specimens of this species I examined at Kew, three possess hollow twigs. On one specimen a small entrance hole is visible. The other nine specimens include sections of twigs, but none of these is hollow. Like other species of *Inga, I. cinnamomea* possesses petiolar/jugal nectaries.

Another possible mimosoid ant-plant is a treelet from Atlantic coastal forests of Brazil currently known as *Pithecellobium incuriale* (true generic position uncertain). A specimen collected in 1986 harbored tiny ants and had a minute entrance hole on a slightly swollen leaf petiole (G. Lewis, pers. comm.).

Eight neotropical papilionoid genera contain possible myrmecophytes, all South American trees. Little is known about any of these. From the hollowed-out twigs and small branches of an unidentified amazonian *Swartzia* sp., Ule (1905) collected ants named by Forel (1904) as a subspecies of *Azteca longiceps* (Dolichoderinae). Wheeler (1942) listed this record, and recorded the same subspecies from *Cordia alliodora* in Bolivia. I found no specimens with hollow twigs or other possibly ant-related traits among the neotropical *Swartzia* at Kew, and Cowan's (1968) monograph mentioned no such structures.

Single South American species of three genera of Sophoreae may be ant-plants. One collection

of *Ateleia pterocarpa* (*Neill 5397,* MO) has hollow, ant-inhabited branchlets, with many entrance holes in the upper parts of inflorescence axes (C. Stirton, pers. comm.). *Alexa cowanii,* from Brazil, has hollow twigs (C. Stirton, pers. comm.). Two separate records exist for *Ormosia macrophylla.* Rudd (1965) mentioned that the collector's notes for one specimen (from Río Pacimoni, Venezuela) stated, "stems hollow; ant infested." J. Zarucchi (pers. comm.) has noted "biting ants" in hollow stems of this species in Vaupés, Colombia (*Zarucchi 1320,* K).

Three genera of Dalbergieae each include one–two South American species that may be ant-plants. Hollow stems inhabited by ants appear to be frequent, perhaps constant, features in at least two species of *Platymiscium, P. stipulare* and *P. ulei* (Spruce, 1908; Ducke, 1925a; Ule, 1905, 1906; Forel, 1904; Wheeler, 1942). In contrast to Ducke (1925a) and other authors, Ducke & Black (1953) mentioned hollow inflorescence axes. Of the three specimens of *P. ulei* at Kew with at least some stem material, only one had hollow twigs. The single specimen of *P.* cf. *stipulare* at Kew lacked hollow twigs. The two Kew specimens of a third species, *P. praecox,* possess hollow twigs. *Pseudomyrmex* species have been most often cited as occupants of *Platymiscium,* but *Azteca* and *Paracryptocerus* (Myrmicinae) have also been taken (Wheeler, 1942). As is true for all but a few leguminous ant-plants, the significance of these ants to the plant, and their host-plant specificity, are open questions.

In an undetermined *Andira* sp. (*Begozo 184,* MO) inflorescence axes and twigs subtending the inflorescences are also swollen, with round to somewhat elliptical entrance holes (C. Stirton, pers. comm.). One species of *Pterocarpus, P. amazonum,* frequently possesses modified structures occupied by ants (Amshoff, 1939; see Fig. 8). Spruce (1908) was the first to describe the swollen inflorescence axes of this plant and noted that they were sometimes, but not always, occupied by ants. Forel (1904) mentioned swollen twigs as well. Of 12 specimens I examined at Kew, in all of the six that included mature inflorescences the axes were swollen; many had numerous ant entrance holes. Of the nine specimens with adequate twig material, seven possessed swollen and hollow twigs. According to Ducke (1925a), these structures are usually occupied by *Pseudomyrmex. Pseudomyrmex sericea* was taken from this plant by Ule (1905; Forel, 1904). According to A. Gentry (pers. comm.), only *Pterocarpus amazonum* in seasonally inundated forest possess ant domatia. These are lacking in the form of this species that occurs in forest on terra firme. The swollen inflorescence axes of these plants are curious myrmecodomatia. If they are no more persistent than inflorescence axes of most plants, they are probably uniquely short-lived among ant-inhabited structures of myrmecophytes. It is also interesting that inflated inflorescence axes arise in single species of these two related genera. In addition to these genera, an undescribed species of *Lonchocarpus* (Millettieae) in Amazonian Peru possesses petiolar domatia similar to those of *Tachigali* (A. Gentry, pers. comm.).

Like most other papilionoid trees, the genera treated above appear to be bereft of extrafloral nectaries. There are no published reports for any of them, and examination of material at Kew of the possibly myrmecophytic species of *Platymiscium* and *Pterocarpus* turned up no indication that they are present.

Three tropical Asian papilionoid genera may include myrmecophytes. Ridder-Numan & Wiriadinata (1985) noted that *Spatholobus oblongifolius* (Millettieae), a large woody climber in northern Borneo, sometimes possesses hollow stems inhabited by ants. Species of *Whitfordiodendron* (*W. nieuwenhuisii* and *W. myriantha;* Millettieae) and *Mastersia* (creepers with hollow stems; Phaseoleae) may also be ant-plants (R. Geesink, pers. comm.).

During the final stages of preparing this manuscript, I encountered two recently published lists of ant-plants, that of Benson (1985) for Amazonian ant-plants and Huxley's (1986) list for the world. These lists do not include the possibly myrmecophytic legumes in the genera *Inga, Swartzia, Ateleia, Alexa, Andira, Pterocarpus, Lonchocarpus, Whitfordiodendron, Mastersia,* and *Spatholobus.* I was unable to locate Bequaert's (1922) mention of a myrmecophytic Asian *Erythrina* cited by Huxley (1986).

THE EVOLUTION OF LEGUME MYRMECOPHYTES

What was the nature of the facultative ant-plant interactions that gave rise to legume myrmecophytes and their often host-specific ant associates? Of those in the Caesalpinioideae and Mimosoideae, all except possibly *Tachigali* and *Sclerolobium* possess extrafloral nectaries, as do their nonmyrmecophytic relatives. In these cases, facultative interactions mediated by extrafloral nectaries appear to have preceded the evolution of myrmecophytes. Extrafloral nectaries have often been cited as a feature of the evolutionary precursors of myrmecophytes (Janzen, 1966; Wilson, 1971).

Possession of extrafloral nectaries, however, is not the whole story. First, examination of surveys of myrmecophytes (e.g., Bequaert, 1922) shows that they have evolved in many plant groups that seem to lack extrafloral nectaries (Zimmermann, 1932) and ant-attractant pearl bodies (O'Dowd, 1982). The production of food directly by the plant does not appear to have been an essential feature of the facultative interactions from which symbiotic ant-plant systems arose. Current information suggests, for example, that the facultative interactions with ants that gave rise to papilionoid myrmecophytes may not have included production of food for ants by plants.

Second, most legume groups with extrafloral nectaries have not given rise to myrmecophytes. In the Mimosoideae, where extrafloral nectaries are extremely widespread in the woody members, ant-plants have evolved in only one, possibly two or three genera. In the genus *Acacia*, ant-plants have evolved twice independently, both times in the subgenus *Acacia*. This suggests that *Acacia* subg. *Acacia* differs in important ways from other mimosoids with extrafloral nectaries.

Evolution of Domatia

I believe that crucial preadaptations in the evolution of myrmecophytes must be sought in other domains. In analyses of the evolution of symbiotic ant-plant mutualisms, too little attention has focused on the evolution of domatia, which distinguish myrmecophytes from other plants. I postulate that relatively few plants possess structures that could serve as protodomatia in facultative interactions with ants. If preadaptations for domatia are rare, then domatia are difficult to evolve. I suggest that myrmecophytes have evolved in some plant groups—but not in others where facultative ant-plant interactions are equally or even more widespread—because these plants possessed suitable morphological traits that could serve as protodomatia. I will illustrate this view with an analysis of the evolution of domatia in legume myrmecophytes. My analysis suggests that myrmecophytes evolved from plants already possessing, at least occasionally, hollow structures that could be inhabited by ants. The ants that occupy these cavities may acquire food from extrafloral nectaries or food bodies, if these are present, but in their absence the ants may tend homopterans in the cavities. Because the cavities serve simultaneously as a nesting site and as a substrate for homopterans, evolution of symbiotic mutualism is not necessarily preceded by production of nectar or ant-attractant food bodies. How do these hollow structures arise? The common structural feature underlying the diversity of myrmecodomatia in the legumes is that they originate in areas where, in their nonmyrmecophytic relatives, there is a local thickening of pith.

The swollen petiole bases of *Tachigali* and *Sclerolobium* offer an excellent example. Many species of these genera are characterized by very long and heavy pinnately compound leaves. Hallé et al. (1978) described a *Sclerolobium* with leaves 2 m long. Such leaves require support. This is provided by an expanded petiole base with hollow-tube construction. I propose that the locally thickened pith at the base of the petioles in proto-*Tachigali* sometimes dried up, providing a cavity that could be entered by arboricolous ants. The size and shape of this cavity could then be modified by selection. Only one other plant genus is recorded in the literature as having petiole-base myrmecodomatia, *Chisocheton* (Meliaceae; Bequaert, 1922). The following quote from Hallé et al. (1978)

suggests that these trees, and their nonmyrmecophytic relatives, may also require support for long, heavy leaves: "In several species of *Aglaia, Guarea,* and *Chisocheton,* the leaves have an indeterminate growth as a result of activity of an apical bud which produces new leaflets over a period of several years, during which time the older leaflets are lost. These leaves may reach a length of 6m."[2]

In *Leonardoxa,* my observations indicate that the evolutionary precursors of myrmecophytes possessed protodomatia, cavities arising fortuitously from structures whose original function had nothing to do with ants. In nonmyrmecophytic populations from noncoastal southern Cameroon, internode apices are slightly expanded. It appears that this expansion provides attachment area and support for the bulky pulvinus of the compound leaf. This expansion is accompanied by a thickening of the pith at the internode apex. In some internodes, the pith is dried up, creating a roughly spherical cavity ca. 4 mm in diameter. I hypothesize that domatia evolved in this species after arboricolous ants began entering these fortuitously produced cavities, occupying the plant more constantly, and providing protection that led to selection for enlarged cavities. A similar scenario seems possible for *Humboldtia.*

In *Acacia* subg. *Acacia,* the joint base of the two stout, thorny stipules provided the area of locally thickened pith that, I presume, occasionally dried up and produced a cavity that could be occupied by arboricolous ants. The presence of large stipular thorns may be the reason myrmecophytes have evolved twice in this subgenus and not in other genera of this subfamily rich in facultative interactions with ants.

Finally, *Platymiscium,* one of few papilionoids frequently occupied by ants, is one of few legumes in which adults bear opposite or verticillate leaves (*Taralea* (Dipterygeae) shares this trait; A. Gentry, pers. comm.). The insertion of two or more leaves at a single node may require local thickening of the stem at the node. It is interesting that stem myrmecodomatia of *Clerodendrum* (Verbenaceae) and of many Rubiaceae, both opposite-leaved families, are often most pronounced at the nodes.

This discussion generates several testable predictions. First, like nonmyrmecophytic *Leonardoxa africana,* other nonmyrmecophytes should be found with cavities created by drying up of locally thickened pith. Second, if such cavities are made available to them, e.g., by drilling of artificial entrance holes, arboricolous ants should enter them. Third, presence of some subset of the arboricolous ant community may result in protection of artificially created myrmecophytes similar to those documented for plants with extrafloral nectaries and for true myrmecophytes.

Evolution of Specificity

Plants bearing extrafloral nectaries but without domatia are frequented by a variety of arboreally foraging, arboricolous or ground-nesting ants. In contrast, most myrmecophytes are associated with fewer ant species, some of them host-plant-specific. The degree of specificity varies among different legume ant-plant systems. The only known case in legumes in which a single species of mutualist ant is restricted to a single ant-plant species is the *Leonardoxa africana* × *Petalomyrmex phylax* system in Cameroon (McKey, 1984). This reflects the taxonomic poverty of a geographically restricted system. Even here, other ants occupy some individual trees, but these appear to be parasites on the system rather than mutualists. In other cases, species-specificity is not absolute. Central American acacia-ants are specialized on the ant-acacia life form, rather than on any particular acacia species, and the converse is also true (Janzen, 1974). *Tachigali paniculata* is inhabited by apparently mutualistic ants of two different genera, *Azteca* and *Pseudomyrmex.*

[2]Indeterminate growth of leaves actually occurs only in *Guarea* and *Chisocheton* (Meliaceae) (J. Fisher, pers. comm.).

These are apparently specific to *Tachigali*, but may occur in species additional to *T. paniculata*. Likewise, *Tachigali* may be associated with other mutualist ants in other parts of its range (Wheeler, 1921b).

How does specificity evolve? Studies by D. Davidson et al. (1988, 1989) of a guild of ant-plants and plant-nesting ants in South America are shedding light on the evolution of specificity in ant-plant symbioses. Previous workers have emphasized pairwise interactions between the plant and its occupant ant(s), and have tacitly assumed that specialization evolves because association with some potential ant partners confers greater advantages to the plant than does association with others, and vice versa. Davidson et al. (1988, 1989) have shown that the selective environment is much more complex than this, and they have highlighted the importance of ant-ant competition for myrmecophytes in determining patterns of host association and, ultimately, the evolution of specificity.

Davidson and coworkers have shown that some ant species are competitively superior to others, and that competitive superiority depends partially on the host plant. Traits of the plant (e.g., trichomes: Davidson et al., 1989) affect the relative success of various ant species, and the plant may evolve characteristics favoring the establishment of ant species conferring greatest benefit. Likewise, ants may evolve characteristics that enhance their success in a particular plant, but not in others. Host specificity may often evolve because an ant species is competitively inferior in all but one host plant (Davidson et al., 1989).

The *Leonardoxa* × *Petalomyrmex* system provides an example of how interactions among different ants inhabiting a plant may drive the evolution of ant and plant traits that lead to or reinforce specificity. In addition to the mutualist ant *Petalomyrmex*, *Leonardoxa* plants are often occupied by another ant, *Cataulacus mckeyi*, whose effect on the plant is strongly negative because it provides no protection and excludes *Petalomyrmex* from shoots it occupies. *Petalomyrmex* has evolved dorsiventrally flattened alates and very small workers, and *Leonardoxa* has evolved long, thin prostomata in which *Petalomyrmex* cuts slitlike holes that allow the large flattened alates (as well as the normally shaped but much smaller workers) to enter but exclude *Cataulacus*. Once the internode matures, the entrance hole cannot be modified. This makes an internode occupied by *Petalomyrmex* impervious to later invasion by *Cataulacus* (McKey, 1984).

The best-known legume ant-plants, the neotropical ant-acacias, are the only myrmecophytes in most habitats they occupy. Coevolution of this system involved only acacias and the arboricolous ant community, and resulted in one group of *Pseudomyrmex* species coevolving diffusely with the ant-acacia life form (Janzen, 1974). Studies of Davidson and coworkers in South American forests suggest possible diffuse coevolution on a much broader scale in habitats rich in myrmecophytes from a variety of plant families. Here, a number of unrelated sympatrically occurring myrmecophytes are occupied by partially overlapping sets of plant-inhabiting ants. This system may provide a great deal of insight into the evolution of highly specialized myrmecophyte systems.

Are any legume ant-plants involved in such facultative, nonspecific, symbiotic mutualisms? Two apparently relatively unspecialized myrmecophytes, *Platymiscium* and *Pterocarpus*, are likely candidates; they occur in habitats containing a variety of other ant-plants, and the few ants reported from them belong to groups usually found in other myrmecophytes. There is reason to suspect that *Tachigali* may have emerged from such a system. Though its mutualist *Pseudomyrmex* are specific to it, according to Wheeler (1921b, 1942), some of their closest relatives are the specific mutualist inhabitants of *Triplaris* (Polygonaceae). Studies in progress on the systematics of *Pseudomyrmex* (P. Ward, pers. comm.) should resolve whether the ants possessed a common ancestor inhabiting two or more incipient ant-plants. It seems likely that in the evolution of ant-plants, diffuse coevolution may have occurred on a scale hitherto unappreciated, and it may be impossible to understand the evolution of legume myrmecophytes independently from that of other, unrelated ant-plants in the same habitats.

Thus my broadbrush review of ant-plant interactions in one of the largest angiosperm families closes on the note that the brush may not yet have been broad enough.

V. Conclusions

The patterns noted in this review lead to hypotheses that can be tested in field studies. The major hypotheses emerging from this study are the following:

1. As in Australia and southern Africa, legumes whose seeds are dispersed primarily by ants will be found in open habitats characterized by frequent fires and possibly nutrient-poor soils. Two outstanding groups in which seed dispersal by ants is likely to be widespread are the genus *Eriosema,* and section *Dispermae* of *Crotalaria* in south-central Africa.

2. Extrafloral nectaries function primarily in the protection of reproductive structures in short-lived Leguminosae and in the protection of vegetative structures, primarily young leaves, in long-lived woody legumes.

3. Patterns in the occurrence among legumes of extrafloral nectaries protecting vegetative structures are related to the phenology of young leaf production, and reflect the interplay of chemical/mechanical, phenological, and biotic defenses in the antiherbivore defense system of the plant. Extrafloral nectaries are most common in legumes with relatively continuous production of young leaves, and less common in those with highly pulsed production of young leaves.

4. In legumes with extrafloral nectaries that have highly pulsed young leaf production, nectaries will be active on mature leaves and may or may not be active on young leaves.

5. The presence of extrafloral nectaries on different organs of the same plant, in addition to distributing ant defense over the plant, may enhance ant defense of each part by increasing the likelihood that ants will be recruited to the plant and by increasing the temporal constancy of nectar production and ant activity on the plant as a whole.

6. The diverse myrmecodomatia of legume ant-plants have one thing in common: they evolved from structures in which there was a local thickening of pith. "Protodomatia" were produced fortuitously in a variety of structures whose original function was not related to interactions with ants. Several plant-architectural features have provided preadaptations for myrmecodomatia. Interactions with facultative ants probably selected for altered shapes and sizes of these structures relatively early in their evolution, as did interactions with obligate ants later.

7. Preadaptations for ant domatia are rare, limiting the frequency with which symbiotic ant-plant mutualisms evolve.

8. Legume myrmecophytes in South America may have arisen in a context of diffuse coevolution from small guilds of unrelated myrmecophytes sharing a group of arboricolous ants nesting in living stems. Diffuse coevolution on a smaller taxonomic scale has occurred in the ant-acacia systems, which are the only myrmecophytes in most habitats where they occur.

ADDENDUM. Since preparation of this paper in 1986, additional information has accumulated. (1) Lersten & Brubaker (1987) presented a review of the occurrence of extrafloral nectaries in Leguminosae. Although their study is not as comprehensive as the review presented here, they reached similar conclusions about the taxonomic patterns: the rarity of extrafloral nectaries in the Papilionoideae, except for tribe Phaseoleae and the genus *Vicia;* the high proportion of Mimosoideae with extrafloral nectaries; and the uncertain extent of occurrence of extrafloral nectaries in the Caesalpinioideae. Their paper includes more detailed information on the structure of legume extrafloral nectaries, particularly those of Phaseoleae, than is presented here. (2) Studies in progress (McKey, unpublished) show that small leaf-surface glands, similar in form to the nectaries of *Leonardoxa africana,* occur in numerous African genera of Detarieae and Amherstieae, and field observations of a few species show that they attract ants. Small foliar nectaries may turn out to be quite widespread in these two tribes. (3) Jugal glands at least superficially similar to those of Mimosoideae are present on herbarium specimens of two African species of *Erythrophleum* (D. McKey, unpublished). This genus is a member of the *Dimorphandra* group considered to be related to the lineage that gave rise to Mimosoideae (Polhill et al., 1981). Studies of fresh or fixed material of *Erythrophleum* and other genera of this group could clarify whether these structures are homologous with the jugal nectaries of Mimosoideae and reflect a very early origin of jugal nectaries in the mimosoid radiation.

Note Added in Proof. Prof. U. Maschwitz, University of Frankfurt, and coworkers have recently discovered two new legume myrmecophytes in southeast Asia, which they will describe in upcoming publications (U. Maschwitz, pers. comm.). *Saraca thaipingensis* (Caesalpinioideae) from West Malaysia has hollow nectaries on young leaves, and the ants tend homopterans (coccids and pseudococcids) inside the hollow stems. *Millettia nieuwenhuisii* (Papilionoideae) from Borneo has hollow stems occupied by a different species of *Cladomyrma*, which tend pseudococcids in them.

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References

Aide, T. M. 1988. Herbivory as a selective agent on the timing of leaf production in a tropical understory community. Nature 336: 574–575.

Amshoff, G. J. H. 1939. On South American Papilionaceae. Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 52: 1–78.

Atsatt, P. R. 1981. Lycaenid butterflies and ants: selection for enemy-free space. Amer. Naturalist 118: 638–654.

Bailey, I. W. 1923. Notes on neotropical ant-plants. II. *Tachigalia paniculata* Aubl. Bot. Gaz. (Crawfordsville) 75: 27–41.

Barton, A. M. 1986. Spatial variation in the effect of ants on an extrafloral nectary plant. Ecology 67: 495–504.

Beattie, A. J. 1983. Distribution of ant-dispersed plants. Sonderbaende des naturwissenschaftlichen Vereins in Hamburg 7: 249–270.

———. 1985. The Evolutionary Ecology of Ant-Plant Mutualisms. Cambridge Univ. Press, Cambridge.

Bell, R. H. V. 1982. The Effect of Soil Nutrient Availability on Community Structure in African Ecosystems. Pp. 193–216 *in* B. J. Huntley & B. H. Walker (editors), Ecology of Tropical Savannas. Springer-Verlag, Berlin, Heidelberg, & New York.

Benson, W. W. 1985. Amazon ant-plants. Pp. 239–266 *in* G. T. Prance & T. E. Lovejoy (editors), Amazonia. Pergamon Press, Oxford.

Bentley, B. L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annual Rev. Ecol. Syst. 8: 407–427.

———. 1981. Ants, extrafloral nectaries and the vine life-form: an interaction. Trop. Ecol. 22: 127–133.

Bequaert, J. 1922. Ants in their diverse relations to the plant world. Bull Amer. Mus. Nat. Hist. 45: 333–583.

Berg, R. Y. 1966. Seed dispersal of *Dendromecon:* its ecologic, evolutionary, and economic significance. Amer. J. Bot. 53: 61–73.

_____. 1975. Myrmecochorous plants in Australia and their dispersal by ants. Austral. J. Bot. 23: 475–508.
_____. 1979. Legume, seed, and myrmecochorous dispersal in *Kennedia* and *Hardenbergia* (Fabaceae), with a remark on the Durian theory. Norwegian J. Bot. 26: 229–254.
_____. 1981. The Role of Ants in Seed Dispersal in Australian Lowland Heathland. Pp. 41–50 *in* R. L. Specht (editor), Heathlands and Related Shrublands: Analytical Studies. Elsevier, Amsterdam.
Bhattacharyya, B. & J. K. Maheshwari. 1971a. Studies on extrafloral nectaries of the Leguminales. I. Papilionaceae, with a discussion on the systematics of the Leguminales. Proc. Indian Acad. Sci. 37B: 11–30.
_____ & _____. 1971b. Studies on extrafloral nectaries of the Leguminales. II. The genus *Cassia* L. (Caesalpiniaceae). Proc. Indian Acad. Sci. 37B: 74–90.
_____ & _____. 1973. Studies on extrafloral nectaries of the Leguminales. III. Mimosaceae. J. Indian Bot. Soc. 52: 267–298.
Bloom, A. J., F. S. Chapin III & H. A. Mooney. 1985. Resource limitation in plants—an economic analogy. Annual Rev. Ecol. Syst. 16: 363–392.
Boehmker, H. 1917. Beitraege zur Kenntnis der floralen und extrafloralen Nektarien. Beih. Bot. Centralb. 33: 169–247.
Bond, W. J. & G. J. Breytenbach. 1985. Ants, rodents and seed predation in Proteaceae. S. African J. Zool. 20: 150–154.
_____ & P. Slingsby. 1983. Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. S. African J. Sci. 79: 231–233.
_____ & _____. 1984. Collapse of an ant-plant mutualism: the Argentine ant *(Iridomyrmex humilis)* and myrmecochorous Proteaceae. Ecology 65: 1031–1037.
Boughton, V. H. 1981. Extrafloral nectaries of some Australian phyllodineous Acacias. Austral. J. Bot. 29: 653–664.
_____. 1985. Extrafloral nectaries of some Australian bipinnate Acacias. Austral. J. Bot. 33: 175–184.
Bower, F. O. 1887. On *Humboldtia laurifolia* Vahl. as a myrmekophilous plant. Proc. Roy. Philos. Soc. Glasgow 18: 320–326.
Brown, W. L., Jr. 1960. Ants, acacias and browsing mammals. Ecology 41: 587–592.
Buckley, R. 1983. Interaction between ants and membracid bugs decreases growth and seed set of host plant bearing extrafloral nectaries. Oecologia (Berlin) 58: 132–136.
Buesgen, M. 1903. Einige Wachstumsbeobachtungen aus den Tropen. Ber. Deutsch. Bot. Ges. 21: 435–440.
Carroll, C. R. 1979. A comparative study of two ant faunas: the stem-nesting ant communities of Liberia, West Africa, and Costa Rica, Central America. Amer. Naturalist 113: 551–561.
Coley, P. D., J. P. Bryant & F. S. Chapin III. 1985. Resource availability and plant antiherbivore defense. Science 230: 895–899.
Cook, A. D., P. R. Atsatt & C. A. Simon. 1971. Doves and doveweed: multiple defenses against avian predation. BioScience 21: 277–281.
Corner, E. J. H. 1949. The Durian theory or the origin of the modern tree. Ann. Bot. (London), New Series 18: 367–414.
Cowan, R. S. 1968. *Swartzia.* Fl. Neotropica 1: 1–228.
_____ & R. M. Polhill. 1981. Detarieae. Pp. 117–134 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.
Czabek, F. 1909. Ueber die Blattentfaltung der Amherstien. Sitzungsb. Akad. Wiss. Wien 118: 201–230.
Daumann, E. 1932. Ueber postflorale Nektarabscheidung. Beih. Bot. Centralb. 49: 720–734.
Davidson, D. W. & S. R. Morton. 1981a. Competition for dispersal in ant-dispersed plants. Science 213: 1259–1261.
_____ & _____. 1981b. Myrmecochory in some plants (F. Chenopodiaceae) of the Australian arid zone. Oecologia (Berlin) 50: 357–366.
_____ & _____. 1984. Dispersal adaptations of some *Acacia* species in the Australian arid zone. Ecology 65: 1038–1051.
_____, J. T. Longino, & R. R. Snelling. 1988. Pruning of host plant neighbors by ants: an experimental approach. Ecology 69: 801–808.
_____, R. R. Snelling, & J. T. Longino. 1989. Competition among ants for myrmecophytes and the significance of plant trichomes. Biotropica 21: 64–73.
Delpino, F. 1886–1889. Funzione mirmecofila nel regno vegetale. Mem. Reale Accad. Sci. Ist. Bologna, Ser. IV. 7: 215–323; 8: 601–650; 10: 115–147.

Dickison, W. C. 1981. Evolutionary Relationship of the Leguminosae. Pp. 35–54 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.
Dominguez, E. 1976. Revision de las especies anuales del genero *Hippocrepis* L. Lagascalia 5: 225–261.
Drake, W. E. 1981. Ant-seed interaction in dry sclerophyll forest on North Stradbroke Island, Queensland. Austral. J. Bot. 29: 293–309.
Ducke, A. 1925a. Plantes nouvelles ou peu connues de la region amazonienne. III. Arch. Jard. Bot. Rio de Janeiro 4: 1–208.
_____. 1925b. As leguminosas do estado do Para. Arch. Jard. Bot. Rio de Janeiro 4: 211–341.
_____ & G. A. Black. 1953. Phytogeographical notes on the Brazilian Amazon. Anais Acad. Brasil. Ci. 25: 1–46.
Dwyer, J. D. 1954. The tropical American genus *Tachigalia*. Ann. Missouri Bot. Gard. 41: 223–260.
_____. 1957. The tropical American genus *Sclerolobium* Vogel Caesalpiniaceae. Lloydia 20: 67–118.
Elias, T. S. 1970. Morphology and anatomy of foliar nectaries of *Pithecellobium macradenium* (Leguminosae). Bot. Gaz. (Crawfordsville) 133: 38–42.
_____. 1980. Foliar nectaries of unusual structure in *Leonardoxa africana* (Leguminosae), an African obligate myrmecophyte. Amer. J. Bot. 67: 423–425.
Escherich, K. 1911. Zwei Beitraege zum Kapitel: "Ameisen und Pflanzen." Biol. Centralbl. 91: 44–51.
Feeny, P. P. 1976. Plant Apparency and Chemical Defense. Pp. 1–40 *in* J. W. Wallace & R. L. Mansell (editors), Biochemical Interaction Between Plants and Insects. Plenum Press, New York & London.
Feinsinger, P., Y. B. Linhart, L. A. Swarm & J. A. Wolfe. 1979. Aspects of the pollination biology of three *Erythrina* species on Trinidad and Tobago. Ann. Missouri Bot. Gard. 66: 451–471.
Figier, J. 1971. Etude infrastructurale de la stipule de *Vicia faba* L. au niveau de nectaire. Planta 98: 31–49.
Forel, A. 1904. In und mit Pflanzen lebenden Ameisen aus dem Amazonas-Gebiet und aus Peru. Zoologisches Jahrbuch. Abteilung Systematik 20: 677–707.
Funke, G. L. 1929. On the biology and anatomy of some tropical leaf joints. 1. Ann. Jard. Bot. Buitenzorg 40: 45–72.
Glyphis, J. P., S. J. Milton & W. R. Siegfried. 1981. Dispersal of *Acacia cyclops* by birds. Oecologia (Berlin) 48: 138–141.
Grear, J. W., Jr. 1970. A revision of the American species of *Eriosema* (Leguminosae, Lotoideae). Mem. New York Bot. Gard. 20: 1–98.
_____ & N. G. Dengler. 1976. The seed appendage of *Eriosema* (Fabaceae). Brittonia 28: 281–288.
Grime, J. P. 1979. Plant strategies and vegetation processes. John Wiley & Sons, Chichester.
Gunn, C. R. 1981. Seeds of Leguminosae. Pp. 913–925 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.
Hallé F., R. A. A. Oldeman & P. B. Tomlinson. 1978. Tropical Trees and Forests. An Architectural Analysis. Springer-Verlag, Berlin, Heidelberg, & New York.
Hocking, B. 1970. Insect associations with the swollen thorn acacias. Transactions of the Royal Entomological Society of London 122: 211–255.
Horvitz, C. C. & D. W. Schemske. 1986. Ant-nest soil and seedling growth in a neotropical ant-dispersed herb. Oecologia (Berlin) 70: 318–320.
Huxley, C. R. 1986. Evolution of benevolent ant-plant relationships. Pp. 257–282 *in* B. Juniper & T. R. E. Southwood (editors), Insects and the Plant Surface. Edward Arnold, London.
Irwin, H. S. & R. C. Barneby. 1981. Cassieae. Pp. 97–106 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.
Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20: 249–275.
_____. 1971. Seed predation by animals. Annual Rev. Ecol. Syst. 2: 465–482.
_____. 1973. Dissolution of mutualism between *Cecropia* and its *Azteca* ants. Biotropica 5: 15–28.
_____. 1974. Swollen-thorn acacias of Central America. Smithsonian Contr. Bot. No. 13: 1–131.
_____. 1975. *Pseudomyrmex nigropilosa:* a parasite of a mutualism. Science 188: 936–937.
Jolivet, P. 1986. Les Plantes et les Fourmis. Un Exemple de Coévolution. Editions Boubée, Paris.
Keeble, F. W. 1895. The hanging foliage of certain tropical trees. Ann. Bot. (London) 9: 59–94.
Keeler, K. H. 1985. Extrafloral nectaries on plants in communities without ants: Hawaii. Oikos 44: 407–414.
Knox, R. B., J. Kenrick, P. Bernhardt, R. Marginson, G. Beresford, I. Baker & H. G. Baker. 1985. Extrafloral nectaries as adaptations for bird pollination in *Acacia terminalis*. Amer. J. Bot. 72: 1185–1196.

_____, R. Marginson, J. Kenrick & A. J. Beattie. 1986. The role of extrafloral nectaries in *Acacia*. Pp. 295–307 *in* B. Juniper & T. R. E. Southwood (editors), Insects and the Plant Surface. Edward Arnold, London.

Koptur, S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. Amer. J. Bot. 66: 1016–1020.

_____. 1982. Biotic Interactions of Costa Rican *Inga* (Fabaceae: Mimosoideae): Pollination Ecology and Antiherbivore Defense. Ph. D. dissertation. Univ. of California, Berkeley, California.

_____. 1984. Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants. Ecology 65: 1787–1793.

_____. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevational gradient. Ecology 66: 1639–1650.

_____. 1986. Interactions between vetches bearing extrafloral nectaries, their biotic protective agents, and herbivores. Abstract, AIBS meetings.

_____ & J. H. Lawton. 1988. Interactions among vetches bearing extrafloral nectaries. their biotic protective agents, and herbivores. Ecology 69: 278–283.

Kuo, J. & J. S. Pate. 1985. The extrafloral nectaries of cowpea (*Vigna unguiculata* (L.) Walp.). I. Morphology, anatomy and fine structure. Planta 166: 15–27.

Kupicha, F. 1976. The infrageneric structure of *Vicia*. Notes Roy. Bot. Gard. Edinburgh 34: 287–326.

Lackey, J. A. 1981. Phaseoleae. Pp. 301–327 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Léonard, J. 1957. Genera des Cynometreae et des Amherstieae africaines (Leguminosae–Caesalpinioideae). Essai de blastogénie appliquée à la systématique. Mem. Acad. Roy. Sci. Belgique, Classe Sci. (8°) 30: 1–312.

Lersten, H. R. & C. L. Brubaker. 1987. Extrafloral nectaries in Leguminosae: review and original observations in *Erythrina* and *Mucuna* (Papilionoideae; Phaseoleae). Bull. Torrey Bot. Club 114: 437–447.

Leston, D. 1973. The ant mosaic—tropical tree crops and the limiting of pests and diseases. PANS 19: 311–341.

Lewis, G. P. & T. S. Elias. 1981. Mimoseae. Pp. 155–168 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Lieberman, D. J. & M. Lieberman. 1984. The causes and consequences of synchronous flushing in a dry tropical forest. Biotropica 16: 193–201.

Majer, J. D. 1979. The possible protective function of extrafloral nectaries of *Acacia saligna*. Mulga Research Centre Annual Report 2: 31–39.

McKey, D. 1974. Adaptive patterns in alkaloid physiology. Amer. Naturalist 108: 305–320.

_____. 1979. The Distribution of Secondary Compounds within Plants. Pp. 55–133 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores. Their Interaction with Secondary Plant Constituents. Academic Press, New York & London.

_____. 1984. Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. Biotropica 16: 81–99.

_____. 1988. Promising new directions in the study of ant-plant mutualisms. Pp. 335–355 *in* W. Greuter & B. Zimmer (editors), Proceedings of the XIV International Botanical Congress. Koeltz Publishers, Koenigstein/Taunus (Germany).

_____. Comparative biology of ant-plant interactions in *Leonardoxa* (Leguminosae: Caesalpinioideae) I. A revision of the genus, with notes on natural history. Ann. Missouri Bot. Gard. (in press).

Metcalfe, C. R. & L. Chalk. 1972 (1950). Anatomy of the Dicotyledons, Volume 1. Clarendon Press, Oxford. [Reprint of first edition, 1950.]

Milewski, A. V. 1982. The occurrence of seeds and fruits taken by ants versus birds in mediterranean Australia and southern Africa, in relation to the availability of soil potassium. J. Biogeography 9: 505–516.

_____ & W. J. Bond. 1982. Convergence of Myrmecochory in Mediterranean Australia and South Africa. Pp. 89–98 *in* R. Buckley (editor), Ant-Plant Interactions in Australia. W. Junk, The Hague.

Morton, J. F. 1983. Rooibos tea, *Aspalathus linearis*, a caffeineless, low-tannin beverage. Econ. Bot. 37: 164–173.

Mueller, P. 1933. Verbreitungsbiologie der Garigueflora. Beih. Bot. Zentralbl. 50: 395–469.

Mueller, R. J. 1988. Shoot tip abortion and sympodial branch reorientation in *Brownea ariza* (Leguminosae). Amer. J. Bot. 75: 391–400.

Nieuwenhuis von Uexkuell-Gueldenbandt, M. 1907. Extraflorale Zuckerausscheidungen und Ameisenschutz. Ann. Jard. Bot. Buitenzorg 21: 195–327.

Noack, F. 1903. Bluetenbiologische Beobachtungen aus Brasilien. Beih. Bot. Centralbl. 18: 112–114.

O'Dowd, D. J. 1982. Pearl bodies as ant food: an ecological role for some leaf emergences of tropical plants. Biotropica 14: 40–49.

Ohashi, H., R. M. Polhill & B. G. Schubert. 1981. Desmodieae. Pp. 292–300 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Ojehomon, O. O. 1968. The development of the inflorescence and extra-floral nectaries of *Vigna unguiculata* (L.) Walp. J. West African Sci. Assoc. 18: 93–110.

Pate, J. S., M. B. Peoples, P. J. Storer & C. A. Atkins. 1985. The extrafloral nectaries of cowpea (*Vigna unguiculata* (L.) Walp.). II. Nectar composition, origin of nectar solutes, and nectary functioning. Planta 166: 28–38.

Polhill, R. M. 1968. Miscellaneous notes on African species of *Crotalaria* L.: 11. Kew Bull. 22: 169–348.

_____. 1971. Genisteae (Adans.) Benth. and Related Tribes (Leguminosae). Ph.D. thesis. University of Reading.

_____. 1976. Genisteae (Adans.) Benth. and related tribes. Pp. 143–368 *in* V. Heywood (editor), Botanical Systematics, Volume 1. Academic Press, London & New York.

_____. 1981a. Adesmieae. Pp. 355–356 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____. 1981b. Loteae. Pp. 371–374 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____ & P. H. Raven, editors. 1981. Advances in Legume Systematics, Parts 1 & 2. Royal Botanic Gardens, Kew.

_____, _____ & C. H. Stirton. 1981. Evolution and Systematics of the Leguminosae. Pp. 1–26 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Putz, F. E. & D. M. Windsor. 1987. Liana phenology on Barro Colorado Island, Panama. Biotropica 19: 334–341.

Rehr. S. S., P. P. Feeny & D. H. Janzen. 1973. Chemical defense in Central American non-ant-acacias. J. Animal Ecol. 42: 405–416.

Rhoades, D. F. & R. G. Cates. 1976. A General Theory of Plant Antiherbivore Chemistry. Pp. 168–213 *in* J. W. Wallace & R. L. Mansell (editors), Biochemical Interaction Between Plants and Insects. Plenum Press, New York.

Rice, B. L. & M. Westoby. 1981. Myrmecochory in sclerophyll vegetation of the West Head, N.S.W. Austral. J. Ecol. 6: 291–298.

_____ & _____. 1986. Evidence against the hypothesis that ant-dispersed seeds reach nutrient-enriched microsites. Ecology 67: 1270–1274.

Richards, P. 1952. The Tropical Rain Forest. An Ecological Study. Cambridge Univ. Press, Cambridge.

Rickson, F. R. 1969. Developmental aspects of the shoot apex, leaf, and beltian bodies of *Acacia cornigera*. Amer. J. Bot. 56: 195–200.

_____. 1975. The ultrastructure of *Acacia cornigera* L. beltian body tissue. Amer. J. Bot. 62: 913–922.

Ridder-Numan, J. W. A. & H. Wiriadinata. 1985. A revision of the genus *Spatholobus* (Leguminosae-Papilionoideae). Reinwardtia 10: 139–205.

Riedl, R. 1979. Order in Living Organisms. A Systems Analysis of Evolution. Wiley, London & New York.

Rippa, G. 1903. I nettarii estranuziali della *Poinciana Gilliesii*. Bull. Orto Bot. Regia Univ. Napoli 1: 431–433.

Ross, J. H. 1981. An analysis of the African *Acacia* species: their distribution, possible origins, and relationships. Bothalia 13: 389–413.

Rudd, V. 1965. The American species of *Ormosia* (Leguminosae). Contr. U.S. Natl. Herb. 32: 279–384.

Sattler, R. 1988. Homeosis in plants. Amer. J. Bot. 75: 1606–1617.

Schemske, D. W. 1983. Limits to Specialization and Coevolution in Plant–Animal Mutualisms. Pp. 67–109 *in* M. H. Nitecki (editor), Coevolution. Univ. of Chicago Press, Chicago, Illinois.

Schimper, A. F. W. 1935. Pflanzengeographie auf physiologischer Grundlage. 3rd Ed. Jena.

Schnell, R., G. Cusset & M. Quenum. 1963. Contribution à l'étude des glandes extra-florales chez quelques groupes de plantes tropicales. Rev. Gén. Bot. 70: 269–342.

Seigler, D. S. & J. E. Ebinger. 1987. Cyanogenic glycosides in ant-acacias of Mexico and Central America. Southw. Naturalist 32: 499–504.

Sernander, R. 1906. Entwurf einer Monographie der europaischen Myrmekochoren. Kongl. Svenska Vetenskapsakad. Handl. 41: 1–410.

Shea, S. R., J. McCormick & C. C. Portlock. 1979. The effect of fires on regeneration of leguminous species in the northern jarrah (*Eucalyptus marginata* Sm.) forest of Western Australia. Austral. J. Ecol. 4: 195–205.

Sherbrooke, W. C. & J. C. Scheerens. 1979. Ant-visited extrafloral (calyx and foliar) nectaries and nectar sugars of *Erythrina flabelliformis* Kearney in Arizona. Ann. Missouri Bot. Gard. 66: 472–481.

Slingsby, P. & W. Bond. 1981. Ants—friends of the fynbos. Veld & Flora 67: 39–45.

Solereder, H. 1908. Systematic anatomy of the Dicotyledons. English edition, translated by L. A. Boodle & F. E. Fritsch. 2 Volumes. Oxford.

Spruce, R. 1908. Notes of a Botanist on the Amazon and Andes. A. R. Wallace (editor). 2 Volumes. London.

Stirton, C. H. 1981a. The *Eriosema cordatum* complex. II. The *Eriosema cordatum* and *E. nutans* groups. Bothalia 18: 281–306.

———. 1981b. Psoraleeae. Pp. 337–343 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Turner, G. W. 1986. Comparative development of secretory cavities in the tribes Amorpheae and Psoraleeae (Leguminosae: Papilionoideae). Amer. J. Bot. 73: 1178–1192.

Ule, E. 1905. Wechselbeziehungen zwischen Ameisen und Pflanzen. Flora 94: 491–497.

———. 1906. Ameisenpflanzen. Bot. Jahrb. Syst. 37: 335–352.

van der Pijl, L. 1972. Principles of dispersal in higher plants. Springer-Verlag, Berlin, Heidelberg, & New York.

Waterman, P. G. & D. McKey. 1989. Herbivory and secondary compounds in rain forest plants: ecological implications and geographical aspects. *In:* H. Lieth & M. Werger (editors), Ecosystems of the World, Volume 14b. Tropical Rain Forests. Elsevier, Amsterdam.

Weiss, F. E. 1908. The dispersal of fruits and seeds by ants. New Phytologist 7: 23–28.

———. 1909. The dispersal of the seeds of the gorse and the broom by ants. New Phytol. 8: 81–89.

Westoby, M., B. Rice, J. M. Shelley, D. Haig & J. L. Kohen. 1982. Plants' Use of Ants for Dispersal at West Head, New South Wales. *In:* R. Buckley (editor), Ant-Plant Interactions in Australia. W. Junk, The Hague.

Wheeler, W. M. 1921a. The Tachigalia ants. Zoologica 3: 137–168.

———. 1921b. A study of some social beetles in British Guiana and of their relations to the ant-plant *Tachigalia*. Zoologica 3: 35–183.

———. 1942. Studies of neotropical ant-plants and their ants. Bull. Mus. Comp. Zool. 90: 1–262.

White, F. 1983. The Vegetation of Africa. UNESCO, Paris.

Wilson, E. O. 1959. Some ecological characteristics of ants in New Guinea rain forests. Ecology 40: 437–447.

———. 1971. The Insect Societies. The Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

Zimmermann, J. G. 1932. Ueber die extrafloralen Nektarien der Angiospermen. Beih. Bot. Zentralbl. 49: 99–196.

APHID–LEGUME INTERACTIONS

M. S. J. Simmonds[1], W. M. Blaney[2], A. N. E. Birch[3] and C. H. Stirton[4]

Abstract

Aphids affect the yield of legume crops, mainly by spreading viral diseases. The role of visual and olfactory cues in the detection of potential host plants by flying aphids is assessed. In probing with the stylets, the aphid encounters nutrients, phagostimulants, and defensive chemicals. The plant may be protected by surface waxes and trichomes, but the principal defense is probably possession of secondary plant compounds. These substances, which may deter probing or prevent sustained feeding, include phenolics, alkaloids, polysaccharides, and other compounds. The occurrence and distribution of such compounds is one of the determinants of aphid–legume specificity, but nutrient levels are also important, as are factors associated with the aphid, such as the species, morph, biotype, size, and age. Many of these factors interact complexly, which influences the ability of the aphid to utilize the plant efficiently.

I. Introduction

The economic impact of the aphid–legume interaction lies not so much in the ability of aphids to decrease the yield of a crop by the amount of plant material they have consumed or by direct mechanical damage, but in their ability to spread viruses. After alighting on a plant, most aphids make one or more brief "test probes" to the epidermis. Test probes are important in the transmission of nonpersistent viruses. The acquisition and innoculation of nonpersistent viruses can result from probes as short as five seconds, with the optimal range 15–30 seconds (Phelan & Miller, 1982). In fact, such legumes as soybean *(Glycine max)* are not colonized by many aphids, but some alate (winged) aphids land on them and test probe before leaving; this spreads nonpersistent viruses (Irwin, 1978).

The cowpea aphid *(Aphis craccivora)* spreads cowpea virus, clover stunt virus, and groundnut mottle virus to many important African crop legumes. This aphid attacks pigeon pea *(Cajanus cajan)* and cowpea *(Vigna unguiculata)* in the semiarid areas of Kenya. In the Eastern Province of Kenya, pigeon pea and cowpea are grown in 115,000 ha and 109,000 ha, respectively, with insect damage in this area being as high as 60% for pigeon pea and 55% for cowpea (Khamala, 1978). In Uganda, the black bean aphid *(Aphis fabae)* can cause up to 90% damage in many legume crops. This species is also one of the most important economic pests in the western world (Nyiira, 1978).

There are about 4,000 species of aphids, mostly in temperate regions (Dixon, 1985). In this paper we outline the various complex interactions between aphids and legumes. Stress is placed on temperate organisms since little is known about tropical aphids and their legume hosts. The scheme we adopt is summarized in Figure 1.

II. Life Cycle

Aphid life cycles may be classified into two types: in one type the aphid spends the autumn, winter, and spring on a woody host plant and the summer on a herbaceous plant (heteroecious life

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, TW9 3DS, England.
[2]Department of Biology, Birkbeck College, Malet Street, London WC1E 7HX, England.
[3]Zoology Department, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland.
[4]The Herbarium, Royal Botanic Gardens, Kew, Surrey, TW9 3AE, England. Current address: Department of Botany, University of Natal, Box 375, Pietermaritzburg, 3200, Republic of South Africa.

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Monogr. Syst. Bot. Missouri Bot. Gard. 29:719–745. 1989.

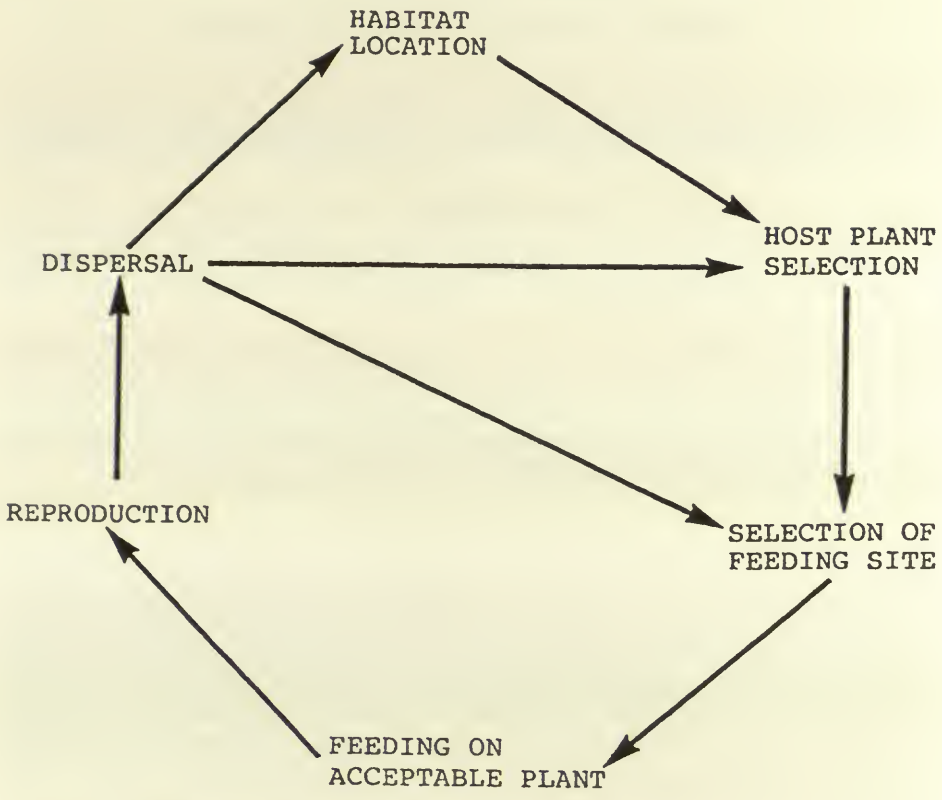

FIGURE 1. Outline of aphid–legume interactions.

cycle); in the other type this regular, seasonal switch in host plant does not occur (autoecious life cycle). In both cases there is a sequence of morphs that differ from each other in morphology and in behavior. Polymorphism has its greatest expression in heteroecious species (Fig. 2). All species of aphids can reproduce parthogenetically, and most can reproduce sexually. Typically there are several generations of parthenogenesis, occurring in spring and summer, between each bout of sexual reproduction which occurs in autumn.

III. Host Specificity

Host specificity is difficult to define in aphids. Normally, very specific insects are restricted to one host species or genus. Host alternation in aphids complicates the picture. Although an aphid may alternate from one host to another at different stages of its life, it may be restricted to a single host species in each stage. Alternatively, an aphid may not alternate hosts in this way and may occur on a wide range of different plants.

Thus 10% of European Aphidinae alternate between plant species, and often the plants are from distinct families. In Britain, 514 species of aphids have been recorded from one plant genus, 70 from two to five genera, 25 from more than five genera within a family, 18 from two to five families, and one from more than six families. This can be summarized to show that more than 90%

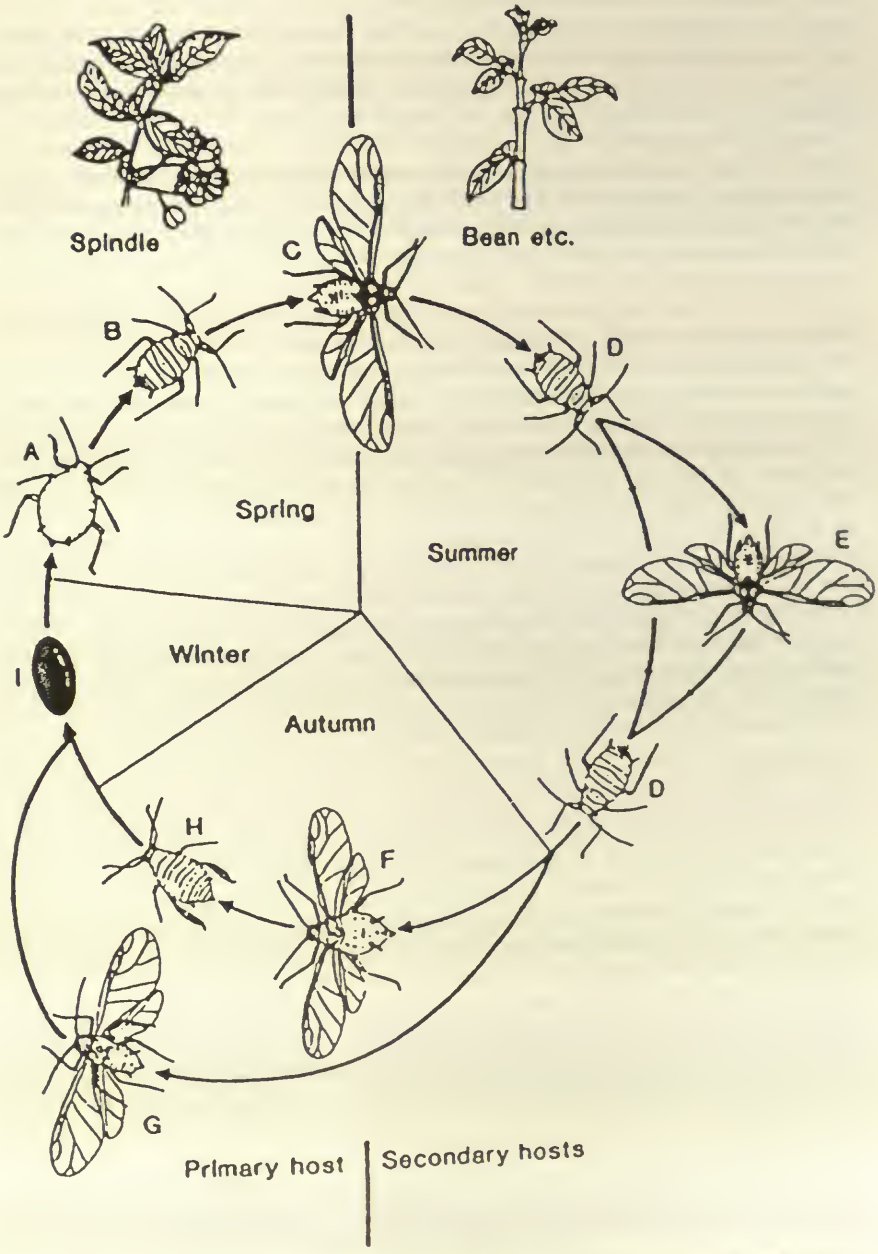

FIGURE 2. Life cycle of *Aphis fabae*. Females (the fundatrices) (A) emerge in spring on the spindle tree (*Euonymus europaeus*) and may or may not produce wingless generations of fundatrigeniae (B) before winged migrantes (C) are produced, which migrate to bean plants and produce wingless exules (D). In summer, winged exules (E) may also be produced. In autumn the exules give rise to winged females, the gynopara (F) and winged males (G). The gynopara are autumn migrants and return to the spindle tree and produce ovipara (H). The winged males join these females, they mate and winter eggs (I) are produced. (Modified from Dixon, 1985.)

of nonalternating aphids and 6% of heteroecious aphid species are specialists, while about 5% of the former group and 63% of the latter feed on more than five plant species (Eastop, 1973). Thus, heteroecious species generally show a greater degree of polyphagy than autoecious species. This is sometimes associated with considerable physiological plasticity and variability in host plant preference.

Another factor that makes it difficult to determine whether or not an aphid is host specific is the problem posed by our current concepts of aphid taxonomy. For example, the different biotypes or land races of the pea aphid *(Acyrthosiphon pisum)* each has specific host preferences (Muller, 1971). In judging specificity it is not always clear whether it is more appropriate to consider the species as a whole or to classify biotypes individually. Similarly, species such as *Myzus persicae* may exhibit wide genetic variability in a population, enabling them to exploit a range of hosts. The specificity of an aphid may also depend on the relative spatial heterogeneity of the habitat, the genetic make up of the aphid, and the interactions between these factors.

Host preference is not always fixed. It can vary among and within individuals in a population with time. The factors that may be involved in this include the physiological condition of the plant, the aphid's physiological condition, and its host history. This variation in preference implies that individual aphids may react in different ways to a host. In fact, biotypes of the pea aphid *(Acyrthosiphon pisum)* can have very different hosts. This is an example of an oligophagous aphid species, which may in fact consist of widely differing biotypes. By investigating the behavior of biotypes of this species at each of the stages in host assessment it might be possible to identify the importance of the genetic makeup of the species and to see how this is involved in the ability of the aphid to overcome the physical and chemical constraints associated with aphid–legume interactions.

The distribution of aphids within a habitat, which in an agricultural context is a crop, may show spatial and temporal differentiation. This will reflect such factors as the occurrence and type of crop rotation, the nature of adjacent crops, the size of fields, and discontinuities in factors influencing landing, settling, and fitness.

IV. Legumes

In this paper we discuss these and other aspects of aphid biology in relation to plants of the Leguminosae. The family has three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae. The caesalpinioids occur in the Americas, tropical Africa, and southeast Asia and are principally tropical or subtropical trees or shrubs. In temperate areas there are some herbaceous species that have descended from woody ancestors (Cowan, 1981). The mimosoids comprise 50–60 genera distributed within the tropics, subtropics, and warm-temperate regions. Two-thirds of the species belong to three genera: *Acacia* (ca. 1,200 species), *Mimosa* (400–500 species), and *Inga* (350–400 species). Many of them are canopy trees but there is a great deal of diversity within the subfamily (Elias, 1981). The papilionoids are made up of approximately 440 genera and 12,000 species. They are widespread in rainforests, deserts, and temperate regions. This subfamily contains the tribe Phaseoleae, which includes some of the most important economic species used as human food, animal fodder, or ornamental plants (Polhill, 1981).

Aphids are not distributed evenly among species in the three legume subfamilies; most feed on species of Papilionoideae. This is partially a reflection of the geographical distribution of the subfamilies. In Europe 106 species of aphids feed on legumes; most belong to the subfamily Aphidinae.

V. Host Plant Stimuli

The stimuli involved in aphid–legume interactions include those acting over long and short ranges to influence detection of the host's habitat, and then detection of the host within that habitat. Once

the aphid is on the plant, stimuli associated with contacting the host will determine whether or not probing occurs. Probing exposes the internal features of the plant that may stimulate the aphid to feed. Feeding indicates host acceptance, but the suitability of the plant is reflected in its ability to support the aphid during prolonged feeding and reproduction.

We highlight some of the constraints that modify or stop this sequence of events. Aphids that alternate among plants show changes in their response to stimuli, which adds to the complexity of the story. This alternating behavior and the fact that the aphids have different morphs that exhibit different behavior and physiological characteristics complicate the study of aphid–legume interactions. The influence of environmental factors, such as temperature, photoperiod, light intensity, and wind speed, will vary in the way they affect the aphid, depending on the stage of the aphid's life cycle. These factors may influence the plant more than the aphid, and it is sometimes difficult to design experiments which resolve this problem.

The relationship of an aphid with its host plant may be considered as a sequential series of interrelated events: detection and location of the habitat and hosts within it (generally from some distance); sampling of the host and assessment of its suitability; feeding on the host; and acceptance of the host as a suitable substrate for development and reproduction. By understanding the mechanism involved in the different levels of this linked process, we may be able to identify the important features in this very complex interaction.

VI. Detection of Hosts from a Distance

Aphids walk about on the surface of plants and sometimes move from one plant to another in that way. Generally, however, invasion of previously uninfested plants is achieved by migratory flights. Because aphids fly slowly (e.g., 1.6–3.2 km/hr) they have little control over the direction of flight: they fly upwards, then downwind, their ground speed being a combination of the wind speed and their own flight speed. They have some control over landing, and color, especially yellow (Kring, 1969), is important in initiating landing behavior. They also respond to differences in the background vegetation or contrasts in color. Van Emden (1972) believed that aphids are cued to approach potential hosts primarily by visual stimulation, although volatiles may be important (Visser & Taanman, 1987) and might increase the responsiveness to visual cues (Kennedy & Ludlow, 1974).

MIGRATION

The aphid's response to these stimuli may be modified by factors not directly involved in host selection, such as migratory behavior. The body of the alate aphid has been described as consisting of two components: the vegetative component, comprising the gut and gonads and involving principally the abdomen, and the combined locomotor/sensory components, involving mainly the head and thorax.

Kennedy (1961) has defined migratory behavior as a movement that is accompanied by an internal inhibition of responses to vegetative stimuli. In fact, a criterion for migration is low responsiveness to vegetative stimuli. Variations in the tendency to migrate are accompanied by variations in searching behavior; the factors that stimulate migration will most likely be those that inhibit host-location behavior.

Migratory flight and host selection have been studied in the black bean aphid, *Aphis fabae* (Kennedy, 1965, 1966; Kennedy & Ludlow, 1974). They found that the stimulation of one behavior inhibited the other. For example, host settling is deterred by elicitation of flight towards a light source. The balance between induction or depression of a behavior depends on the internal states of each system and on the sensory inputs received by the aphid (Kennedy, 1966). The stimuli

associated with migration (e.g., short-wave radiation emitted by the sky) are not the same as those involved in targeted flight (e.g., long-wave radiation emitted by the ground), but the aphid's response to these stimuli will partly depend on its recent experience. Thus, when an aphid has flown for some time it is more responsive to stimuli from the ground and is more prone to land again than it was immediately after the start of flight.

There is evidence that aphids can distinguish between the sky and the ground on the basis of wavelength (above or below 500 nm) (Kennedy, 1961) and that they can distinguish between soil and plants. *Aphis fabae* can distinguish colors; yellow and red decrease flight activity in migrating aphids, thereby increasing the probability of landing (Kring, 1969).

Although the dispersal flight of aphids is largely passive, the behavior of a teneral or predispersal aphid is different from that of a nondispersal aphid. The dispersing aphid will walk up the plant onto the tips of the leaves. During this movement it rarely probes the substrate with its rostrum, whereas a nondispersing aphid will settle on the plant and may begin to probe in the vicinity of the landing site. Before take-off, a dispersing aphid is more responsive to stimuli that promote dispersal, such as crowding or low nutrient quality, than it is to similar stimuli after landing following a migratory flight.

PLANT DISTRIBUTION

Once an aphid has landed, it will take off if the plant is unsuitable and will land again shortly afterwards. Aphids become aggregated by a process of trial and error on host plants.

The turbulence set up by breaks in vegetation may be important in concentrating aphids within an area (Eastop, 1973). Trap data have shown that *Myzus persicae* and *Aphis fabae* alight with similar frequency on equal-sized plants of many species. However, they only accumulate on host plants because they leave nonhosts more rapidly than they do hosts (Müller, 1962). The extent to which the aphid's behavior is switched from migration to settling is influenced by the relative quality of the cues it receives, differentially promoting alternative behaviors. Cues from different host plants vary in their capacity to alter the aphid's behavior.

The stimuli involved in host location also vary with the pattern of occurrence of the host, which can have a temporal and spatial distribution. This distribution could be a factor in the evolution of host specificity and in the development of the aphid's life cycle. In tropical areas plant diversity is high, and therefore the apparency of any one species is low. This is thought to be one of the reasons that most tropical aphids are polyphagous. Plants in the tropics have little temporal variation, which means that the aphids, once on the host, do not have to alternate between hosts. However, since individual plants of a given species may be infrequent and widely scattered, most aphids need to be able to exploit more than one host species. These aphids have to respond to very different environmental pressures than those encountered by temperate aphids.

In temperate areas where the diversity of plants is lower, the aphids are more oliphagous or are monophagous. This is especially true where agricultural practices have further decreased plant diversity. The plants in temperate areas often have a temporal or seasonal element to their distribution, and aphids have developed the ability to alternate between seasonally abundant plants. The physiological changes in the aphids' response to the stimuli from these alternate hosts are a complex puzzle of which we have only assembled a few pieces.

VII. Identification of Hosts on Contact

After landing on a plant, the aphids walk over the surface. Some species antennate, others start to probe the surface with their stylets.

INSECT SENSORY RECEPTORS

There is evidence that the sensilla on the antenna respond to short-range volatiles, and work by Shambaugh et al. (1978), Bromley et al. (1979), and Bromley & Anderson (1982) suggests that aphids use their antennae to "taste" the plant. Some sensilla, at the tip of the proboscis, are thought to be tactile. They provide the aphid with information about pubescence, veins, and other surface irregularities (Tjallingii, 1978).

A report by Tarn & Adams (1982) suggested that there are chemoreceptors at the tip of the proboscis, which could be used to detect interactions between the chemicals present in aphid saliva and the plants. However, in an earlier paper, Tjallingi (1978) found no evidence for proboscis chemoreceptors in the aphids he examined. During the initial investigation of the plant surface there is little or no stylet penetration, yet within about 60 seconds the aphid is able to assess the plant's suitability. During this probing many aphids exude some saliva onto the plant's surface (Harris, 1977; Pollard, 1977). Van Emden (1972) stated that by responding to plant surface chemicals they can reject a plant after only a ten-second probe.

PLANT TRICHOMES

Klingauf (1972) listed the most important features of the plant's surface in relation to host selection as: waxes, hairiness, and secondary plant compounds. He thought secondary plant compounds were important in restricting the aphid's host range. It is uncertain how many of these compounds can be perceived by the aphids on contacting the plant, but those contained in the trichomes or those that are volatile may be contacted by the aphids.

Trichomes provide a good physical defense and are probably the most important morphological feature of the leaf involved in aphid–leaf contact interactions. If the pubescence is dense and the hairs are of sufficient length and appropriate orientation, the aphid is unable to contact the plant's surface to feed. The level of pubescence on *Phaseolus vulgaris* has been correlated with mortality in the bean aphid (Johnson, 1953). Trichomes within the legumes are varied; unicellular, multicellular, nonglandular, and glandular with many structural variations, for example, straight, spiral, hooked, simple, peltate, or stellate (Baudet, 1974).

Nonglandular trichomes confer physical resistance against aphids. Poos (1929) and Poos & Smith (1931) showed that there was a correlation between insect resistance and pubescence in soybeans. Levels of pubescence ranged in different genotypes from dense (31 hairs/mm^2), through normal (8.1 hairs/mm^2), to sparse (2.6 hairs/mm^2). Bernard & Singh (1969) showed that the difference between these genotypes of soybean could be related to one gene at one of four distinct loci. Hairs can prevent feeding or trap aphids. Recent work has shown that the density of the hairs is not always the key factor that stops feeding and that their orientation is important (cf. Kennedy, 1986).

The correlation noted above between aphid mortality and hair density is due to the type of hair and in particular hair shape. The hairs on many species of *Phaseolus* are hooked (Baudet, 1974; Lackey, 1978), and aphids are caught by their tarsal claws as they walk on the leaf. The aphid is thus held to the leaf, and its jerky movements may cause a hooked hair to penetrate its cuticle; death may result from this damage or from starvation. Such mortality is common in the first and second instars (Johnson, 1953).

Glandular hairs have been shown to contain tannins, essential oils, and sesquiterpenes, and some produce exudates that repel aphids. The occurrence of deterrent compounds in exudates on the surface of the plant is a good way of decreasing herbivory, especially if the compounds are not continually present on the surface but are released when an insect contacts and breaks the glandular hair containing the chemical. The best-studied example of this is found within the Solanaceae. The wild potato, *Solanum berthaultii*, has two types of hairs. Type A hairs are glandular and when broken exude a fluid that can trap an aphid. Type B hairs are likewise glandular and when broken

release (E)-β-farnesene, an aphid alarm pheromone, which initiates an alarm response in the aphids, making them depart from the foliage (Gibson & Pickett, 1983). Thus, these trichome exudates are very important modifiers of aphid behavior. If the type B trichomes are removed from potatoes, the aphids probe sooner after landing on the plant. If the plant has only type A trichomes, the aphids are not deterred from feeding, but if the type B exudates are transferred to plants lacking the B-type trichome, the aphids are deterred from feeding (Lapointe & Tingey, 1984).

Other exudates, such as the alkaloids nicotine, veratrine, tomatine, acotine, and delphinine, can act as gustatory deterrents or toxins (Levin, 1973). These chemicals are perceived by the chemoreceptors on the aphid's antennae and mouthparts. Bromley & Anderson (1982) have shown that the primary rhinaria on the aphid's antennae are sensitive to a wide range of volatiles, especially terpenes. The sticky glandular hairs of *Bolusafra bituminosa* and *Rhynchosia bullata* are so dense on young growing shoots that they trap even large spiders. Both these species appear to be unaffected by aphids in either the wild or in greenhouses (Stirton, pers. obs.) Within the Leguminosae, *Rhynchosia leucoscias, R. viscosa, R. transvaaliensis, R. bullata,* and *Boulsafra bituminosa* all have bulbous-based trichomes that break open on contact and trap insects, including aphids (Stirton, pers. obs.).

Trichomes within a plant species vary in type and density with geographical distribution presumably due to physical components of the environment. This variation may be a by-product of some selection process, or it may reflect direct selection, perhaps by aphids (Levin, 1973).

The presence of trichomes has also been shown to be linked with virus transmission. The green pea aphid *(Acyrthosiphon pisum)* spreads potato virus yellow (PVY). If the pubescence delays probing activity, the ability to transmit the virus is reduced. If the aphid is prevented from probing for an hour there is a 60% decrease in its ability to infect a new plant with PVY (Bradley, 1954).

PLANT VEINS

The position of veins is another important physical feature of leaf surfaces. When *Aphis fabae* alights on a leaf it keeps walking until it encounters an obstacle, such as a vein. This type of activity is partially responsible for aphids clustering at certain points, where they begin to probe. Lowe (1967) has shown that different feeding sites are selected by different species, the distribution being a result of their probing behavior. The feeding position can also be influenced by weather conditions and the density and proximity of leaves (Dixon, 1985). Another potentially significant anatomical feature of the leaf in deterring probing by aphids is the high concentrations of crystals found near veins (see Zindler-Frank, 1987, for a detailed review of the distribution of calcium oxidate monohydrate crystales in legumes).

PLANT CUTICLE

Since the aphid has to penetrate the plant's cuticle before feeding, the chemistry of the cuticle could be important. The plant surface consists of cutin, wax, pectin, and cellulose. A thick cuticle is thought to prevent early instars of *Aphis fabae* from feeding (Davidson, 1923), but Zettler et al. (1969) could find no correlation between the probing behavior on beans by *A. craccivora, A. gossypii, A. spiraecola,* and *Myzus persicae* and such anatomical features of the leaf as cuticle thickness.

The cuticle of *Vicia faba* contains 20 different n-alkanes. These waxes are very common and similar in plants, but differ in their relative proportions. In *V. faba* the n-alkanes C_{27} H_{56} (5%), C_{29} H_{60} (19%), and $C_{31}H_{64}$ (43%) are the most abundant and the proportion of the C_{29} alkane is thought to be important in influencing the ability of the black bean aphid *(Aphis fabae)* to discriminate

between its host *V. faba* and nonhosts, such as cabbage. In cabbage, C_{29} alkane is present in a high proportion and deters *A. fabae* (Klingauf, 1982, referring to work by Nocker-Wenzel).

VIII. Factors that Influence Probing

The term probing, used to describe investigation of a plant by aphids, has no precise, consistent meaning. The term is applied to a relatively cursory, superficial examination of the plant surface and to a more prolonged examination that involves penetrating the plant and sampling its internal constituents.

If the aphid is not deterred from the leaf by contact or olfactory stimuli, it will stop walking and often start to probe. The two events rarely occur together. Indeed, Ibbotson & Kennedy (1959) suggested that they are two mutually exclusive activities controlled by the central nervous system. A similar situation occurs with flight and walking in the alate aphid. In both cases, it is difficult to show whether a particular feature of the environment is stimulating one activity or inhibiting the other, and generally diverse stimuli are involved. While probing, the aphid protracts its rostrum, with its antennae pointing forward and vibrating. It then thrusts repeatedly at the plant surface with its rostrum. Once probing starts, the antennae are laid back. If it is unable to obtain a firm hold to exert pressure on the plant, the aphid will move, trailing its rostrum along the substrate, and renew probing at a new site. As the stylets penetrate the surface, the labium becomes displaced as the head approaches the surface.

Aphid Mouthparts

The aphid's mouthparts are well adapted for probing (Fig. 3). They consist of two pairs of slender chitinous stylets that penetrate the plant. Between the opposed inner pair are two canals, an anterior food canal, and a smaller, posterior salivary canal. The diameter of the food canal is 1–2 μm and that of the salivary canal is 0.2–0.4 μm (Auclair, 1963, 1964). The stylets are enclosed within the labium, which is shortened by telescopic action during feeding. The cibarial-pharyngeal pump within the aphid's head contributes to the passage of food through the pharynx into the esophagous. The importance of this in determining the rate of feeding is discussed below. Many aphids probe until the phloem is reached and feed from that tissue, but some species can also feed from xylem or from mesophyll (Pollard, 1973).

Aphids secrete two types of saliva: a water fluid containing enzymes and a viscous fluid that gels to form a sheath round the stylets and extends to the feeding site. The feeding site is usually discovered by histological examination of plant tissue on which aphids have been feeding, and many authors have described where the stylet sheaths have terminated. Sorin (1966) showed that with *Myzus persicae* feeding on three different legume hosts the rate at which the stylet sheaths terminated in phloem was 97.9%, 98.1%, and 77.6%. For *Aphis craccivora* on five hosts, the figures for terminating in the phloem were 96.6%, 75.5%, 100%, 89.4%, and 100%.

The depths of penetration are determined partially by the lengths of the stylets. In *Aphis fabae*, the stylet length is 420 μm, of which 315 μm can be used for probing (Berlinski, 1965). The structure of parenchyma of *Vicia faba* could aid in directing the stylets of *A. fabae* to the phloem (Muller, 1958). In general, probing behavior may be modified by physical and chemical features acting together or singly.

Experimental Techniques

There is often a good correlation of probing behavior on plants with that on artificial substrates. These artificial substrates can be readily controlled and manipulated to investigate the effects of

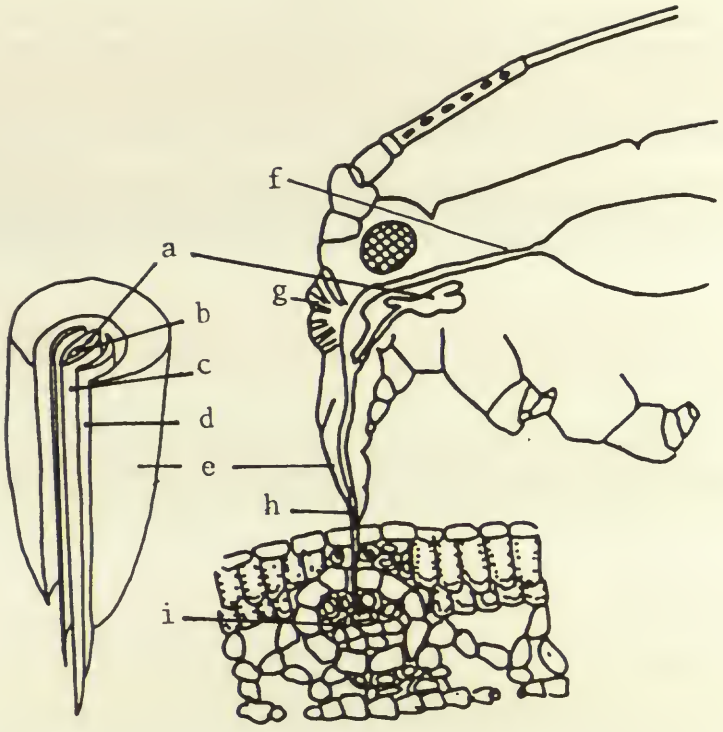

FIGURE 3. Aphid mouth parts a–h. (a) Salivary gland, (b) food canal, (c) maxilla, (d) mandible, (e) labium, (f) gut, (g) food pump, (h) stylets, (i) vascular bundle of plant.

chemical and physical foliar features. An electrical recording technique developed by McLean & Kinsey (1968) has yielded valuable information on probing behavior. McLean & Kinsey attached an aphid to a fine silver wire electrode, and a 60 Hz AC voltage was applied to the leaf or feeding substrate by another wire electrode. The electrodes were then connected to an amplifier, and when the aphid's stylets started to penetrate the substrate, an electric circuit was completed. The activity of the aphid modulates the applied voltage, and the wave forms produced are then correlated with the position of the stylets in the tissue using histological techniques. Using the alfalfa aphid *(Therioaphis maculata)* the experimenters divided the behavior into three stages: 1) salivation wave, in which the sheath material is formed in the initial probe to the location of the vascular bundle; 2) X-wave form, which corresponds to the penetration of the sieve elements in phloem; and 3) ingestion wave form, which occurs on the withdrawal of sap from the sieve elements.

This type of analysis has been expanded by Tjallingii (1978), who showed that there were at least six different electrical patterns. Some of these occur simultaneously and are superimposed. He used radio isotope experiments to correlate the electrical patterns with the aphid's behavior. The results confirmed that some patterns were better correlated with saliva secretion or with ingestion than others. Although there are still problems in interpreting the signals, this has proved to be a useful technique in studying interactions of the aphid with host and nonhost plants.

The electrical waveforms recorded result largely from changes in electrical resistance occurring in the aphid–plant preparation and the effect of that resistance on the applied voltage. The changes in resistance appear to be determined by different mechanisms: valve mechanisms that can open and close the food or saliva canals; fluctuating concentration of electrolytes in the food or saliva;

position of stylets (for example, whether they are entering a more or less conductive element of the plant); capillary diameter of the stylet canals; fluid velocity through the canals; and membrane potentials from plant cells or from the aphid's gut, heart, brain, or muscles. As Tjallingii (1985) stated, an understanding of the electrical properties of the preparation is essential for a correct interpretation of the electrical patterns. Correlation of the electrical patterns with behavior is somewhat limited in that our knowledge of the penetration behavior is incomplete. It is not always known whether the stylets pass between or through cells, cell walls, protoplasts, or vacuoles, or when sap samples are taken, and from which part of the plant tissue. However, recent evidence (Kimmins, 1986) suggests that for some aphids penetration of the leaf may be intercellular, intracellular, and intramural (i.e., between cell walls). Results of this study suggest that occasionally the contents of some parenchyma cells are ingested. The presence or absence of gustatory stimuli (sugars, amino acids, or specific secondary compounds) may direct the aphid's probing towards the phloem.

Passage of the stylets through plant tissue has been studied with stylet-cutting techniques and with light and electron microscopy. This type of investigation provides data that show that host plants are associated with long probes and fluid uptake and that nonhosts have short probes and little fluid uptake. The question arises as to what factors influence stylet penetration and how they are perceived.

PLANT PHENOLICS

Probing behavior by aphids has been shown to be influenced by phenolics (Dreyer & Jones, 1981) polysaccharides (Campbell et al., 1986), and alkaloids (Corcuera, 1984; Schoonhoven & Derken-Koppers, 1976) and by amino acids and sugars. Jordens-Rottger (1979) found that *Aphis fabae* was strongly attracted by low-molecular-weight phenolic substances in a bioassay, while less reaction was shown to flavonoids. Bioassays have shown that extracts of host plants without phenolics were less attractive than those with them.

PLANT ALKALOIDS

Many legume alkaloids have been shown to influence feeding. Smith (1966) has reported that the quinolizidine alkaloid sparteine acts as a feeding stimulant for the broom aphid *(Acyrthosiphon spartii)*. Winks et al. (1982) found that *Aphis cytisorum* prefers plants with a low quinolizidine content, although this aphid can sequester and metabolize these alkaloids. Dreyer et al. (1985) found that castanospermine but not swainsonine was active against the pea aphid. The activity of castanospermine could be due to its ability to inhibit aphid hydrolases, β-glucosidase, and α-glucosidase. Dreyer et al. (1985) also suggested that castanospermine may inhibit trehalase, but this was not shown in their experiments. Dreyer et al. (1985) found that the pea aphid was able to colonize the locoweed *(Astragalus lentiginosus)*, and that in the honeydew from these aphids there was a substantial amount of swainsonine. This compound, a known mannosidase inhibitor, is water soluble, transported in the phloem, and excreted by the aphid.

PLANT POLYSACCHARIDES

Campbell et al. (1986) showed that plant polysaccharides affect the probing behavior of some species of aphids and that this class of biopolymers could play an important role in aphid–legume interactions. Aphids contact these compounds when probing, especially when penetrating cell walls. In fact, they have more chance of contacting polysaccharides than some of the allelochemics

that may be unavailable to the aphids because they are contained within cells or cell vacuoles. The aphid's saliva contains polysaccharases with a broad-spectrum depolymerase activity. The monophagous and oligophagous aphids appear to show greater sensitivity to polysaccharides than do the polyphagous species. Of the polysaccharides tested by Campbell et al. (1986), all generally elicited a negative response in *Acyrthosiphon pisum*. The aphids may also contact these compounds during the preprobing activity when many species show brief and repetitive periods of less than two minutes of salivation and imbibition followed by extravation. During this activity, salivary enzymes, including carbohydrase, polysaccharase, polyphenol oxidase, and peroxidase, are discharged from the stylets. The time when the saliva is produced and the duration of secretion varies between species. Kloft (1960) found that *Myzus ascalonicus* secretes saliva only during stylet insertion, which occurs between six and eight minutes after the start of probing, and when the stylets are being withdrawn. The aphids that feed on parenchyma *(Adeles, Chermes, Dreyfusia)* secrete saliva more or less continually during probing.

APHID SALIVA

The two types of saliva produced have different functions. The viscous material forms the stylet sheath and is thought to contain pectinases. Its viscous gelling quality is considered to be the result of enzymatic oxidation to quinones of lipoprotein sheath precursor (Miles, 1964). The watery saliva is an alkaline secretion containing hydrolytic enzymes. Especially important are the pectinases and cellulases that assist in stylet penetration and the phenolases that counteract the plant's defense reactions. The secretion also aids digestion of food and lubrication of the stylets. The presence of pectinase is thought to be significant in the way aphids are able to penetrate the plant (McAllen & Adams, 1961). Species without pectinase generally penetrate intracellularly whereas those with pectinase penetrate intercellularly. Therefore pectinase could be aiding penetration by the hydrolysis of the pectin middle lamella.

Amino acids and amines are present in the saliva. These are thought to be partially responsible for eliciting some defense responses in plants and for the types of injury seen in plant tissue after aphid penetration. They are also involved in gall formation, polyploidy, and plasma streaming (Anders, 1958; Marek, 1961). The concentration of free amino acids in the saliva may be relatively high, for example, 7% in *Viteus vitifolii* (Anders, 1958). The types of amino acids in different species vary and are influenced by diet.

PROBING BEHAVIOR

McLean & Kinsey (1968) found major differences in the probing behavior of *Acyrthosiphon pisum* on nonhosts (e.g., lettuce) and host (e.g., *Vicia faba*). On nonhosts, the aphid is stimulated to ingest sap from epidermal, subepidermal, or mesophyll parenchyma cells; on hosts they ingest from phloem. On nonhosts they make more probes than on hosts.

McLean & Kinsey (1984) have investigated which sensilla contact plant constituents and believe that the initial gustation does not occur until imbibed fluids contact the precibarial chemosensilla. Stimulation then either results in ingestion or extravation, the behavior resulting from cues that elicit further probing or from distasteful substances. Contacts with monosaccharides generally stimulate feeding. Some monosaccharides and oligosaccharides could be in the tissue as a result of the salivary enzymes breaking down the plant matrix polysaccharides. These breakdown products then act as phagostimulants. Campbell et al. (1986) found that *Acyrthosiphon pisum* did not respond to acetylated pectins, which may reflect the fact that the host legumes of this species do not contain these pectins. The monosaccharides arabinose and glucose were stimulants to this aphid.

While probing, aphids are able to minimize the intake of deleterious compounds by withdrawing

their stylets from mesophyll cells or their vacuoles, or by not ingesting the contents. The duration of the probing phase varies greatly and depends on the palatability status of the plant and the physical structure of its cells and tissues. Data suggest that during probing aphids detect and are influenced by nutrients and allelochemics, tissue pH, physical features, and tissue pressure (Pollard, 1973; Klingauf, 1982).

In host plants, *Myzus persicae* takes one minute to penetrate the epidermis, three to five minutes to pierce the epidermal layer and a few mesophyll cells, and more than 15 minutes to reach the phloem. The depth at which the aphid will reach the phloem and its speed will vary due to chemical and physical barriers. The length of the aphid's stylets will also influence penetration, and this varies between instars within a species. On *Vicia faba, Aphis fabae* does not feed while penetrating the epidermis and cortex, and takes from 25 minutes to an hour to reach the phloem and commence feeding. Two to three hours are required for 82–87% of a population of the vetch aphid *(Megoura viciae)* to reach the phloem (Eharhardt, 1961). When feeding on pea leaves, *Acyrthosiphon pisum* can penetrate up to 5 μm of the epidermis in ten seconds, 8 μm in 30 seconds (50% of the stylets are in the parenchyma), and 18 μm in 60 seconds, by which time the majority of the stylets are in the parenchyma. It takes about another four minutes to reach the phloem (Nault & Gyrisco, 1966). In another study with *Acyrthosiphon pisum,* this time feeding on stems, the aphid took one minute to penetrate 0.37 mm, two minutes to penetrate 1.16 mm, and ten minutes to penetrate 1.78 mm. The type of tissue the stylets have to penetrate will influence probing time. The ratio of intercellular to mixed path penetration varies with the type of host (Sorin, 1962, 1966). Variation is thought to be related to tissue compactness which restricts the passage of stylets. Stylet paths through loose tissue are much straighter than through dense tissue.

Cell structure is most likely important in modifying the passage of the stylets through the tissue, but what guides the stylets to the phloem is not known unequivocally. The pH gradient of tissues could be important. In sugar beet the pH of parenchyma is 5.9 and that of phloem is 7.5. Marek (1961) has shown that aphids can detect and discriminate between different levels of pH. For example, *Myzus ascalonicus* prefers solutions in the range pH 6.2–8.4.

VIRUS TRANSMISSION

Probing behavior is important in spreading viruses. When on nonhost plants, aphids are restless, and their probing activity increases. This will ensure the rapid speed of, for example in cowpea, the nonpersistent cowpea aphid-borne mosaic virus (CAMV) (Atiri et al., 1984). Atiri et al. studied the settling behavior of the alatae and apterae of *Aphis craccivora* in relation to the spread of virus, and the effect of acquisition of the virus by aphids from aphid-resistant cowpea lines on the spread of virus infection. They found a positive correlation between alate count and incidence of CAMV in cowpeas. However, aphids cannot acquire CAMV from aphid-resistant cowpea plants as quickly as from aphid-susceptible or aphid-tolerant plants, so that the spread of virus from the resistant plants is reduced. Watson & Plump (1972) have observed that nonpersistent viruses can be transmitted by probes of a few seconds, such as occur on aphid-resistant plants. However, with persistent viruses and some semipersistent ones, there is often a positive correlation between the time taken for the aphid to acquire the virus and subsequent transmission: these viruses are not transmitted during short probes.

IX. Factors that Influence Feeding

By the term feeding we refer to the more or less sustained intake of plant fluid that occurs once the aphid has accepted a plant. It is not easy to determine when this has started and to differentiate it from probing behavior: together they characterize a large part of the aphid's interactions with the

plant. It has been proposed that some of the aphid's ingestion receptors respond to phagostimulants or feeding deterrents and control feeding initiation, while other ingestion receptors respond to specific nutrient requirements and control prolonged feeding (Kennedy, 1985).

The chemical composition of the sap is the most important factor influencing sustained feeding, but additional factors are important in determining whether the aphid will feed and the rate of feeding. The literature contains many measurements of feeding rates that need to be standardized before comparisons can be made. Such comparisons are further hampered by enormous variation in experimental circumstances, such as: the aphid morph studied; the time of year; the species, cultivar, and age of plant; and the physiology of the plant. Banks & Nixon (1958) recorded that *Aphis fabae* on *Vicia faba* ingested at a rate of 200 mg/hr over 12–16 hours feeding, which is 59% of body weight per hour, whereas Banks & Macauley (1964) reported the same species ingesting 30 mg in 21 days. The latter authors also recorded nymphs of the same species ingesting 3.5–4.5 mg of sap in seven days. On an artificial diet, *Acyrthosiphon pisum* ingested 12–16 mg/hr (Auclair, 1965). The amount of honeydew produced has often been used to measure feeding rate. This has been estimated by counting the number of honeydew drops produced over a given time period and multiplying by the volume of a standard drop. Auclair (1959) stated that the frequency and rate of excretion of an aphid are generally proportional to the susceptibility of the pea it is feeding on. Busgen (1981), in a study involving eight species, reported that the number of drops produced in a given time varied from 1.7 to 20, and that the diameter of the drops varied from less than 1 mm to 1.5 mm. The rate of excretion depends on the life stage of the aphid, which morph it is, the plant species and variety, its growth stage, and various environmental factors. Another complication is illustrated by *A. pisum*, which in a study remained for 3.5–5.8 hours in the feeding posture on a susceptible plant without feeding (Auclair, 1959). Such behavior results in very variable excretion rates within a population.

The details of how aphids feed are not known completely; whether they feed passively or actively and what is the role of the plant sap pressure in feeding. Kennedy & Fosbrooke (1973) stated that the aphid regulates its food intake by varying either the duration of feeding bouts or the interval between them. The longevity of an aphid can be increased by increasing the pressure of a deficient diet but not if the diet is complete. This suggests that pressure may influence the intake, but only when feeding is suboptimal.

The aphid's mouthparts include four stylets which taper to a fine point (0.04 μm). Because of their shape, any pressure applied to them is maximized at the distal tip, making them well adapted for penetration into plant tissue. The maxillary stylets join the precibarium, a canal formed by the union of the epipharynx and hypopharynx, which links the food canal of the maxillary stylets and cibarial pump. The precibarial valve at the junction between the hypopharynx and epipharynx is closed by cuticular tension. The precibarial valve and the cardiac valve produce a unidirectional, discontinuous pumping system (McLean & Kinsey, 1984) during active and passive feeding.

In active feeding, for fluids to move into the alimentary canal a pressure difference must be created between the source of the fluid and the anterior section of the alimentary canal. The uptake of fluids is regulated by the contractions of the various muscles associated with the valves. Not all fluids that enter the stylets are ingested: some can be forced back through the stylets. Fluids within the stylets will contact the chemosensilla. Fluids that are not phagostimulants or that induce an antifeedant response will be rejected.

Passive feeding has often been thought to be associated with sap pressure (McLean & Kinsey, 1968). Wright & Fisher (1980) suggested that aphids feeding on phloem may not need to pump fluids actively into the alimentary canal. Banks & Nixon (1958) showed that in *Aphis fabae* the uptake and flow rate of fluids under the hydrostatic pressures found in the sieve tubes may be controlled primarily by the regulatory function of the cibarial pump. Thus the cibarial pump can be involved in active and passive feeding.

It is clear from the available data that feeding is influenced significantly by a number of factors related to the aphid and to the plant, which for clarity may be considered separately.

TABLE 1. The distribution when feeding of six aphid species on *Vicia faba* (data from Lowe, 1967).

Aphids	Percentages feeding on:				
	Midrib	Large veins	Other veins	Lamina	Margin
Megoura viciae	37.9	50.6	22.5		
Aphis fabae	29.4	55.4	15.2		
Acyrthosiphon pisum	3.9	38.9	54.4	2.8	
Aulacorthum solani	0.9	20.8	75.7	1.6	1.1
Myzus persicae	0.7	11.2	67.5	6.3	14.2
Myzus ornatus	—	2.6	24.6	7.6	65.2

SPECIES OF APHIDS

The feeding pattern often differs significantly among species. Polyphagous species generally feed on highly nutritious food as they are very responsive to levels of sugars and amino acids. The high nutrient quality of their food could compensate for the metabolic expense of detoxifying or excreting potentially harmful compounds where such compounds occur in host plants but do not inhibit feeding. Monophagous species are usually more sensitive to levels of allelochemics than nutrients and are often deterred by them, whereas some require the presence of specific allelochemics to trigger or sustain feeding. Some monophagous aphids can detoxify such compounds, whereas others sequester them for use in their own defense. For example, the vetch aphid sequesters plant chemicals that render it distasteful and toxic to predatory ladybird beetle larvae (Dixon, 1973). Phloem sap has a poor nitrogen content and aphids often require symbionts to supplement their diet.

These factors are reflected in the interactions between aphids and legumes. *Aphis fabae* is one of the most economically important aphid pests in Europe. It attacks more than 33 plant families. It causes considerable damage by feeding on the plants and to a lesser extent by transmitting bean yellow mosaic virus (BYMV). *Acyrthosiphon pisum* also damages legumes directly, and indirectly by spreading BYMV and bean leaf roll virus (BLRV). This oligophagous species attacks nine legume genera. Although *Megoura viciae* is not a major pest of *Vicia faba,* it can transmit BLRV and BYMV.

Lowe (1967) studied the feeding behavior of six aphid species on *Vicia faba* and found differences in feeding site, as reflected by their settling position on the plant. He divided the leaf into five parts: the basal two-thirds of the midrib; the large veins visibly raised above the leaf surface, including the distal third of the midrib; the other veins visible in the leaf lamina; the central area of the lamina, which has no veins; and the lamina margin, i.e., the narrow strip about 2.5 mm wide beyond the terminal forks of the visible veins. The distribution of feeding aphids in these areas is shown in Table 1.

Myzus viciae and *Aphis fabae* differed less from each other than from the other species. The central lamina was the least-preferred feeding site. The position of the probes within the leaf varied among species. The adult *A. fabae* probes mainly into the larger veins (50 μm). In *Myzus* species the probes of adults were distributed in either the spongy mesophyll or veins, in the ratios of 1:2 in *M. persicae* and 2:1 in *M. ornatus.* In all species, the nymphs probed in the mesophyll more frequently than did adults. Lowe (1967) recorded that in most cases, the probes were intercellular. The sheaths were found to follow the contours of cells, adhering to the inner surface of the epidermis until a mesophyll cell was reached, then passing over the surface of cells. Probes were not found to enter any mesophyll cells. More recent evidence indicates that some aphids sample cell contents during penetration by intracellular probes (Kimmins, 1986).

The preferred feeding site for an aphid on a common host can vary. On *Vicia faba, Aphis fabae* colonizes leaves and stems equally but strongly prefers the lower surface of the leaf (Salyk & Sullivan, 1982). In the same study, when *A. fabae* and *Acyrthosiphon pisum* were raised together,

TABLE 2. Sap uptake by *Acyrthosiphon pisum* and *Aphis fabae* (data from Auclair, 1978).

Aphids	Plants	Fluid production (μl/hr)
Acyrthosiphon pisum	*Pisum sativum*	
(3rd instar	'Perfection'	0.07 (honeydew)
to apterous		0.12 (stylet sap)
adults)	'Melting Sugar'	0.04 (honeydew)
		0.08 (stylet sap)
Aphis fabae	*Vicia faba*	0.2 (stylet sap)

Aphis fabae changed preferences, preferring leaves to stems, reflecting interspecific competition. The vetch aphid, *Megoura viciae*, feeds on *Vicia faba* and strongly prefers stem feeding sites (Llewellyn & Qureshi, 1979). Llewellyn & Qureshi (1979) have shown that when *Aphis fabae*, *M. viciae*, and *Acyrthosiphon pisum* are reared on seedlings of *V. faba*, the nymphs of *M. viciae* grow five times larger than *A. fabae* in a development time only two days longer. The larval growth efficiencies are only 10% greater in *M. viciae*, and adult reproduction is only 5% greater; *A. pisum* is similar to *M. viciae*.

SPECIES OF PLANTS

A study involving 22 wild *Vicia* species and cultivars of *V. faba* has shown that the reproductive potential of *Aphis fabae, Acyrthosiphon pisum,* and *Myzus viciae* was influenced by the plants (Birch & Wratten, 1984; Holt & Birch, 1984). *Acyrthosiphon pisum* out-performed the less oligophagous *M. viciae* and the more polyphagous *Aphis fabae*. They recorded a great range of antibiotic resistance within *Vicia* and thought that nymphal survival rate was the most strongly affected factor. During field trails in 1980 and 1981, the close wild relatives of the faba bean *(Vicia narbonensis)* and *V. johannis* were found to be more resistant to *Aphis fabae* than was the crop plant *V. faba* (Birch, 1985).

The species of plant may influence the rate at which the aphid is able to feed. Turgor pressure in plants affects sap uptake, as does sap viscosity and the length and diameter of aphid stylets. The sap uptake by *Acyrthosiphon pisum* and *Aphis fabae* varies on different cultivars of pea *(Pisum sativum)* as seen in (Table 2).

McLean & Kinsey (1968) suggested that there are different mechanisms involved in the process of host plant selection undertaken by *Acyrthosiphon pisum*. They proposed four behavioral differences to explain why the aphids do not survive on *Vigna unguiculata* but do survive on *Lactuca sativa* (Compositae). These are:

1. On *V. unguiculata* they ingest sap from the epidermis, subepidermis, or the mesophyll.
2. On *V. unguiculata* they probe the phloem but rarely ingest.
3. On *L. sativa* they ingest fluid from sieve elements.
4. On *V. unguiculata* they make many unsuccessful probes.

AGES OF APHIDS

The feeding rates of aphids decline during the post-reproduction phase (Banks, 1965). Moulting also influences the rate of feeding and excretion. *Aphis pisum* ceases to produce honeydew for five to eight hours during each nymphal molt.

AGES OF PLANTS AND LEAVES

The physiological age of the leaf influences feeding. The nutrient quality of phloem changes with the plant's age and with the season. Growing and senescent leaves are richer in nitrogen than mature leaves. The age of the plant is important in determining where *Aphis fabae* may feed. This species is very polyphagous and sensitive to the plant's nutritive state. It changes its feeding site from shoot tips early in the growing season to mature leaves, then to flowers and pods as the plant develops (Birch, 1985). The plant's nutritional state is affected by flowering time, development rate and structure. In some aphid-resistant legumes, a multistemmed growth form spreads the plant's growth between many shoots and fruits so that individual sites available to aphids are numerous, but each has relatively low nutritional value (Müller, 1958; Birch, 1985).

The rate of photosynthetic assimilation varies with age and can be taken as an index of leaf fitness. The peak density of aphids occurs on leaves with peak fitness. This would be expected of insects that remain on one plant for a long time, but more mobile insects would be more likely to move from plant to plant to maximize feeding potential (Hartnett & Bazzaz, 1984).

SIZES OF APHIDS

There is a positive relationship between aphid size and feeding rate. Within a species successive instars feed on larger veins of a leaf. In general, large aphids are more fecund than small aphids (Dixon, 1976), and this affects the feeding potential of a population. Size is a relative term and is influenced by birth weight and the nutritional quality of the plant. Although it certainly reflects the aphid's performance on a plant, it is not always a very good indicator of aphid performance. Therefore, it is better to use measurements of the reproductive rate and the relative growth rates when assessing such things as antibiotic resistance.

The size of the aphid influences the size of its progeny so that in many species large individuals produce large progeny. Dixon & Dharma (1980) looked at the correlation between aphid size and fecundity in the apterous exulates of *Aphis fabae*. They found a high correlation between adult weight and number of embryos and between adult weight and the number of well-developed embryos. The embryos in large aphids were larger than those in small ones. In *A. fabae*, large size and high temperature are associated with a short prereproductive delay; small size and low temperature result in a long delay. However, Llewellyn & Brown (1985) obtained data from over 90 aphid species living on about 200 different plant species and discerned a negative relationship between adult weight and embryo number. There was no seasonal component to this relationship, although the number of well-developed embryos with pigmented eyes increased as the weight of alates increased from June onwards. The number of embryos per unit adult body weight declined as adult size increased. Apterous aphids always had more embryos than alates did per body weight.

APHID MORPHS

The stage in the life cycle is an important determinant, not only of the current damage to the host plants, but also of the potential of an aphid population to cause local or more widespread damage. Food uptake is greater in apterous aphids than in alates (Raccah & Tahari, 1971), therefore, factors affecting change of morph can have a profound, long-term influence.

A number of stimuli are involved in determining the production of particular morphs, including changes in temperature, crowding, and the quality of the host plant (Lees, 1966). Apterae of *Megoura viciae* reproduce parthenogenetically under long day conditions and apterous or alate

development is determined immediately before birth. In *M. viciae* an "interval timer" is thought to delay production of ovipora (Lees, 1966). Many aphids produce alates when their host becomes unsuitable for continued colonization (Sutherland, 1967, 1969). However, the factors affecting such changes are complex. The response could be due to interactions among aphids since a poor diet increases their restlessness. Alternatively, some studies have shown that alates are produced when their performance is optimal on high-quality hosts (Parry, 1977).

When considering the influence of the plant on morph determination the term "nutrient quality" should be defined. Nutrients and/or allelochemics could exert the major influence via the diet and nutritional state of the aphid. The alternation of the type of progeny is thus controlled by both intrinsic and extrinsic factors. The aphid is responding to the influence of light and to information from sensilla associated with the rostrum, tarsi, antennae, and gustatory receptors in the hypopharynx (Wensler & Filshie, 1969).

The factors that influence morph production vary among species. For the cowpea aphid *(Aphis craccivora)* starvation results in production of more apterae (Johnson, 1966). Sutherland (1969) found that isolated apterae of the green biotype of *Acyrthosiphon pisum* fed on mature bean leaves produced alates within 24 hours. The ability to produce different morphs could be seen as a means of exploiting the host plant. Apterae of *Megoura viciae* are very active and gravid females disperse from densely populated plants, although localized colonies could develop on individual plants. To colonize aphid-free sites the dispersal response would decline in favor of a reproductive response, resulting in an increase of apterous progeny.

The fecundity of *Aphis fabae* apterae on the spindle tree differs from those on their summer hosts. These differences are associated with genetically programmed differences in the number of ovarioles in individuals of each generation. The aphids feeding on spindle trees are larger and have more ovarioles than those on other plants. This variation is as large as that seen in the apterous viviparous morphs. Size has been correlated with fecundity and this could be due to small aphids dying early in life, therefore having a short reproductive life compared with the larger aphids. Additionally, large aphids could have better-developed gonads. The physical and biological environments influence aphid fitness, and consequently some have a varied spatial and temporal distribution. *Aphis fabae* can remain on the spindle, its primary host, if predators are absent, with no decrease in reproductive potential (Dixon, 1971). Crowding can influence the behavior of a morph (Kidd & Cleaver, 1984), leading to more interactions among individuals, which promotes the production of alates in many species. In *A. fabae,* Shaw (1970) described three types of alates: migrants which flew before reproduction; flyers which fly after they deposit one or a few nymphs; nonflyers which do not require a flight before reproduction. Shaw suggested therefore that flight was a reflection of the degree of migratory urge possessed by an alate. However, as a wide range of interforms exist between apterae and alatae, variations in morphology and physiology occur. Shaw (1970) suggested two factors that determine whether an alate will become a migrant, flyer, or nonflyer: (1) size and (2) urge to take off mediated by such stimuli as crowding during its development.

Kidd & Cleaver (1984) asked whether the increased urge to take off reflects an increased tendency to undertake migratory as opposed to trivial flights, and whether flyers are less migratory than migrants. Kennedy (1961) regarded the suppression of vegetative reflexes, such as reproduction, as a migratory characteristic, but failure to reproduce before takeoff does not in itself indicate migratory potential. Johnson (1969) proposed the oogenesis-flight syndrome, which compared the relative development of the flight apparatus in migratory aphids with that of the reproductive system in nonmigratory aphids. He found in migratory aphids that energy was channeled into the production of wings, wing muscles, and a large fat body at the expense of embryos, whereas in sedentary aphids, the reverse was true. In addition, migrants have a higher proportion of their dry weight consisting of fat. Cockbain (1961) showed that in *Aphis fabae* glycogen was used up during the first hour of flight, after which fat reserves were utilized. The size of the fat reserve influences the capacity to migrate, as aphids with a higher fat content flew for longer than those with a lower fat content.

THE APHID BIOTYPE

Acyrthyosiphon pisum comprises a number of biotypes, each of which is adapted to a different range of legumes (Müller, 1962). The occurrence of different biotypes can create problems for workers on plant resistance. There is a need to test new cultivars on several natural aphid populations rather than on a restricted biotype, as a new resistant genotype may arise rapidly in the indigenous population when a new crop is introduced.

In central Europe, *Acyrthosiphon pisum* appears in several biotypes on herbaceous legumes. Müller (1980) placed them into two complexes. One attacks mainly peas, is green, and has alates of both sexes. They are further characterized by producing alates throughout the year. In autumn they migrate to *Vicia villosa* and other perennial wild species of *Vicia*. Members of the second complex have apterous males and sexuparae. Müller worked on three biotypes of *A. pisum*, two of which live on peas and reject alfalfa while the third lives on alfalfa and rejects peas. One of the biotypes on peas is reproductively isolated from the alfalfa biotype by post-mating mechanisms, thus this biotype could be in fact a distinct subspecies.

The nutritional requirements of two biotypes of *Acyrthosiphon pisum*, collected from Ile-aux-Coudres and Saint-Jean, differ but both biotypes require ascorbic acid, folic acid, niacin, calcium pantothenate, pyridoxine, and thiamin (Auclair & Boisvert, 1980). The biotype Saint-Jean has a consistently higher development rate than the biotype Ile-aux-Coudres. This difference could result from the diverse genetic makeup which favors a better development of the biotype Saint-Jean.

In Australia, Hughes & Bryce (1984) found that two biotypes of *Acyrthosiphon pisum* coexist in the same population of one host plant, lucerne. One of the biotypes is resistant to the fungal pathogen *Erynia* sp., which was thought to be important in the establishment of the two biotypes. However, they continue to coexist when the pathogen is absent. The resistant biotype has a greater intrinsic rate of increase at high temperatures and a reduced propensity to form wings in response to crowding. These factors helped to build up the resistant population relative to the susceptible one in a crop. The pathogen (*Erynia* sp.) selectively killed the susceptible individuals. The resistant population increased at a faster rate with the summer temperature and emigrated less when the population became crowded. But as this biotype does not migrate as well as the susceptible biotype, it is restricted in its distribution. It is of interest that no sexual form of the aphid exists in Australia (Hughes & Bryce, 1984).

THE QUALITY OF FOOD

The aphid is greatly affected by the quality of the food it ingests, and this had been well investigated. Particularly important are sugars and amino acids (Mittler, 1970), which can act synergistically, but antibiotics also have an important influence (Mittler, 1971). Experiments have shown that *Acyrthosiphon pisum* finds amino acids without the functional group more attractive than those with phenolic groups.

The concentration of nonprotein amino acids in wild *Vicia* leaves and stems is correlated with antibiotic resistance to *Aphis fabae* (Holt & Birch, 1984), but the concentration of protein amino acids is not (Holt & Wratten, 1986). The phenolic L-Dopa is present in *V. faba* leaves but its concentration is not correlated with resistance. In fact, it is an arrestant and feeding stimulant to this aphid (Jordens & Klingauf, 1977). The low level of honeydew of *A. fabae* feeding on resistant varieties could be the result of better utilization of sap ingested, rather than the level of amino acids in the phloem. The low honeydew amino acid concentration on resistant *V. faba* might be associated with smaller leaf veins on resistant cultivars, which are also more prone to aphid-induced plant disruption. This results in the aphids moving from these veins to the larger ones, which tend to have a lower nitrogen content (Holt & Wratten, 1986).

Klingauf (1972) gave data on the importance of 22 amino acids to *Acyrthosiphon pisum* sepa-

rately and together. He measured the duration of the first and second probes and the time between the probes. At high concentration, the amino acids had a repellent effect (brief probes), but after dilution the aphids made longer probes. He found that he could divide the amino acids into three groups. The most acceptable were the amino monocarboxylic acids (seven subgroups) followed by the amino dicarboxylic acids, then the amino acids. The most preferred amino acids were aspartic acid, isoleucine, valine, and leucine.

The composition of plant amino acid affects the feeding of biotypes of *Acyrthosiphon pisum* differently. Biotype "C" requires arginine, histidine, lysine, methionine, threoine, and tryptophan; biotype "J" requires the same as "C" plus phenyalanine and valine (Srivastava et al., 1985). Experiments by Srivastava et al. (1983) have looked at the role of these amino acids to see if they are phagostimulants or nutrients or both. They suggested that symbionts could be important in supplying aphids with missing amino acids, cholesterol, free fatty acids, monoglycerides, and diglycerides. Mittler (1971) suggested that the symbionts of *Myzus persicae* supplied the aphid with most of its amino acids when these were not available in its food. These studies have shown that different biotypes have different amino acid requirements. Variation in nutrients and secondary compounds occurs among and within plants. Increase in the concentration of amino acids results in an increase in the population of aphids.

Polyphagous species are more sensitive to amino acid changes than monophagous species. Many polyphagous species are deterred by high levels of secondary plant compounds, whereas a single compound in the host of a monophagous species might elicit a positive response (Van Emden, 1972), and help indicate an appropriate plant. Coumarin plays an important part in host plant selection by the cowpea aphid (*Aphis craccivora*)(Mansour et al., 1982). Phenolics are important to *Aphis fabae* (Jordens-Rottger, 1979), which prefers extracts containing phenol, hydroquinone, quaiacol, and dehydrocaffeic acid to those without these chemicals.

Schoonhoven & Derken-Koppers (1976) studied the effects of allelochemics on food uptake and survival of *Myzus persicae* and concluded that its polyphagy is due to its freedom from deterrence by some chemicals and avoidance of others. Workers in China found that with the same aphid species reared on tobacco, feeding was reduced by the following compounds in order of effectiveness: nicotine, phlorizin, gossypol, tomatine, malic acid, inuline, sinigrin. Inulin, malic acid, and sinigrin did not affect development or reproduction (Qin Junde & Ke Lidao, 1984).

X. Plant Acceptability

The fact that an aphid gets so far as to feed on a plant does not guarantee that its stay on the plant will be prolonged or that the plant will prove suitable for production of offspring and establishment of an aphid colony. Clearly, there will be degrees of suitability, which will be reflected in degrees of "fitness" of the aphid. This relationship can be measured in a number of ways, using criteria such as preference and abundance (Bond & Lowe, 1975), fecundity and development (Holt, 1981), mean relative growth rate (Van Emden, 1969), and longevity—in general, the factors that appear in life table studies.

In addition to describing the conditions of the aphid, these criteria reflect the degree of susceptibility or resistance of the plant. That aspect of the aphid–plant interaction is usually placed into one of three categories: tolerance, antibiosis, or antixenosis. However, the mechanisms in the aphid–legume interaction that result in the interaction being placed into one of these categories are often imperfectly understood. In studies on the alfalfa aphid *(Therioaphis maculata)* tolerance and antibiosis have been recorded (Howe & Smith, 1957). The antibiosis can often result in death after 24–72 hours, which could be due to a toxin or to nutritional deficiency. In this case, observations showed it to be due to starvation. Initially the aphids would settle on the plant, but after about one to four hours they began to wander. This type of activity was recorded over an 8–36-hour period.

Investigations of the salivary sheaths showed that on the resistant plants the stylets rarely terminated in the phloem.

HOST PLANT RESISTANCE

The work by Bond & Lowe (1975) on the resistance of field beans to *Aphis fabae* confirms the earlier report (Müller, 1961) that the cultivar 'Rastatt' is resistant. However, these workers could not correlate any growth patterns in this or other field beans with resistance. Nor could they correlate the content of soluble nitrogen with resistance. They also showed that the resistance of *Vicia narbonensis* to *A. fabae* differed from the resistance of *Vicia faba* to *Myzus persicae*, as the latter species can colonize *V. narbonensis*. The *V. faba* lines 14 and 27/1/3, which were derived from 'Rastatt', were also resistant, indicating that this resistance is heritable.

The mechanism of resistance to aphids by these plants is still not fully understood. The amino acid profiles of leaves before aphid attack are very similar in resistant and susceptible *Vicia faba* cultivars (Holt & Birch, 1984). However, there is evidence that aphids can manipulate their host plants to obtain optimal quantities of nutrients, including carbohydrates and amino acids. Hussain et al. (1973) showed that aphid infestations can cause changes in host plant hormone levels, which in turn increase levels of free amino acids available for feeding. These changes are induced either by a component of the aphid's saliva or as a result of damage to plant cells. Consequent increases in abscisic acid levels suppress plant protein synthesis, increasing the pool of free amino acids. Such changes can decrease the prereproductive period of *Aphis fabae* and can increase its fecundity by a factor of ten (Scheurer, 1976). More recently, Poehling & Morvan (1984) studied chemical changes in resistant and susceptible cultivars of *V. faba* that occur rapidly after feeding by *A. fabae*. On leaves of a susceptible cultivar, carbohydrates accumulated more rapidly, and several amino acids, including glutamine and methionine, increased in quantity, whereas in a resistant cultivar there was a large increase in asparagine.

An important aspect of "fitness" is the effectiveness of the aphid in using material derived from the plant. This is influenced by the metabolic capability of the insect and by the composition of the plant material.

HOST UTILIZATION

Llewellyn & Qureshi (1978) have studied the energetics and growth efficiency of *Aphis fabae* on different parts of the broad bean plant. When the aphids are feeding on young stems the energy intake is 122% compared with that on mature stems. They also found that the efficiency of conversion to biomass is similar. When feeding on the young stems there was a 50% increase in reproduction, but these aphids were 17% less efficient than those feeding on mature leaves and stems.

Llewellyn & Leckstein (1978) studied the energy budget of *Aphis fabae* when reared on artificial diet and on *Vicia faba*. They overcame some of the difficulties in looking at food efficiencies by using radio isotopes. On the plant, the aphids had a growth efficiency of 50%, whereas those on the diet showed only 20% efficiency. On the plant the larval growth rate was 0.05 mg/day; on the diet it was 0.037 mg/day, a decline of 34%. Akey & Beck (1971) found that *Acyrthosiphon pisum* on an artificial diet had a growth rate of 0.16 mg/day compared with 0.21 mg/day in plant-reared aphids. This type of study highlights a major difficulty in monitoring aphid performance on modified diets when these diets are so different from the aphid's normal host. The pattern of food utilization is also different. The main differences are in the conversion of food to body mass and the amount lost in honeydew. The amount lost in respiration and excuvia is similar in both types of food. Llewellyn & Qureshi (1979) compared the energy budgets of *Aphis fabae*, *Acyrthosiphon*

pisum, and *Megoura viciae*. They found that *M. viciae* grows five times larger than *A. fabae* in a development time of only two days longer. *Acyrthosiphon pisum* is of a similar size to *M. viciae*. The proportion of ingested food that is utilized during respiration is much higher in *Acyrthosiphon pisum* than in *Aphis fabae*.

Metabolic constituents of the plant that influence its acceptability to the aphid include the nutrients and the allelochemicals. Sucrose is found in the sap at concentrations ranging from 5–25% (Auclair, 1964). Part of it is absorbed in the gut and the remainder eliminated or condensed to the trisaccharide melezitose (Srivastava & Auclair, 1962) or to oligosaccharides, which reduces the gut osmotic pressure (Basden, 1968). Auclair et al. (1957), studying the importance of amino acids, monitored the aphid population on a range of pea varieties. The cultivar ('Perfection') with the highest aphid population had the highest level of amino acids. Fourteen to 25 amino acids were detected and the percentage of each varied within a plant and among cultivars. The ones that were present at higher concentrations in the susceptible cultivar, 'Perfection', were arginine, aspartic acid, leucine, lysine, threonine, tyrosine, and valine.

Srivastava et al. (1985) reported that diet uptake by *Acyrthosiphon pisum* was lowest on diets lacking amino acids. For first through third instar larvae, uptake was high when the amino acid concentration was 3.5% and for fourth instars and adults at 2.5%. Over a 24-hour period they took two to five times more of a diet with amino acid than one without. The amount consumed will of course vary with instar, and first through third instars take two to four times less than fourth instars or adults. In this experiment methonine had a phagostimulatory effect. The results also indicate the synergistic effect of amino acids with sucrose. Leckstein & Llewellyn (1973), working with *Aphis fabae*, considered histidine and methionine to be essential dietary components for protein synthesis but that they were not acting as phagostimulants, whereas alanine and proline did act as phagostimulants. Mittler (1970) found that in *Myzus persicae* food uptake was reduced by 50% if methionine was not present. The absence of cysteine or histidine resulted in a decrease of 15–30%. Harrewijn & Noordink (1971) found similar results, which also included isoleucine.

Of the amino acids required for protein synthesis, some act as phagostimulants. The amino acids can stimulate uptake by their presence, as with alanine or proline, or by their absence, as with histidine or methionine. Aphids reared on diets deficient in proline, alanine, and serine produce offspring almost the same size as those on the complete diet, which indicates that these amino acids are not essential dietary requirements (Leckstein & Llewellyn, 1973).

Studies by Srivastava et al. (1983) showed that, of the 23 amino acids in the pea aphid's diet, only lycine, homoserine, leucine, threonine, and valine were phagostimulants and nutritive; alanine, γ-amino-buytric acid, phenylalanine, and tryptophan were mainly phagostimulants; arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, proline, and tyrosine were mainly nutritive. It is possible that the presence and relative concentrations of one or more of these amino acids in the sap may increase or decrease feeding by the pea aphid and thus influence the aphid–legume interaction. Aphids also ingest and metabolize dipeptides and, as they have proteases in their salivary glands, they could partially digest protein in the sieve tubes. Sources of nitrogen other than the free amino acids should not be overlooked in such investigations.

XI. Summary

The detrimental effect of aphids on the yield of legume crops results mainly from the spread of virus diseases by the aphids. Flying aphids detect potential host plants by means of visual cues, and olfactory cues may also be important. After landing, the aphid assesses the suitability of the plant by superficial probing with the stylets. In so doing it will encounter nutrients, phagostimulants, and defensive chemicals. The plant may be protected by surface waxes and trichomes of various types but in many cases the principal defense is probably the possession of secondary plant compounds. These substances may deter further probing or prevent sustained feeding. They include phenolics,

alkaloids, polysaccharides, and other types of compounds. The occurrence and distribution of such compounds within plants and among plant species is one of the determinants of specificity in the aphid–legume interaction. Nutrient levels are also important, especially to polyphagous species of aphids. Factors associated with the aphid that affect the relationship with plants are its species, morph, biotype, size, and age. The suitability of a plant for the establishment of an aphid colony is governed by a complex interaction of many of the above factors, which influence the "fitness" of the aphid and its ability to utilize the plant efficiently.

References

Akey, D. K. & S. D. Beck. 1971. Continuous rearing of the pea aphid, *Acyrthosiphon pisum*, on a holidic diet. Ann. Entomol. Soc. Amer. 4: 353–356.

Anders, F. 1958. Aminosauren als gallenerregende Stoffe der Reblaus (*Viteus (Phylloxera) vitifolii* Shimer). Experientia 14: 62–63.

Atiri, G. I., E. J. A. Ekpo & G. Thottappilly. 1984. The effect of aphid-resistance in cowpea on infestation and development of *Aphis craccivora* and the transmission of cowpea aphid-borne mosaic virus. Ann. Appl. Biol. 104: 339–346.

Auclair, J. L. 1959. Feeding and excretion by the pea aphid, *Acyrthosiphon pisum* (Harr.) (Homoptera: Aphididae), reared on different varieties of peas. Entomol. Exp. Appl. 2: 279–286.

———. 1963. Aphid feeding and nutrition. Ann. Rev. Entomol. 8: 439–490.

———. 1964. Recent advances in the feeding and nutrition of aphids. Canad. Entomol. 96: 241–249.

———. 1965. Feeding and nutrition of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphidae) on chemically defined diets of various pH and nutrient levels. Ann. Entomol. Soc. Amer. 58: 855–875.

———. 1978. Biotypes of the pea aphid, *Acyrthosiphon pisum*, in relation to host plants and chemically defined diets. Entomol. Exp. Appl. 24: 212–216.

——— & J. M. Boisvert. 1980. Besoins qualitatifs en vitamines hydrosolubles chez deux biotypes du puceron du pois. *Acyrthosiphon pisum*. Entomol. Exp. Appl. 28: 233–246.

———, J. B. Maltais & J. J. Cartier. 1957. Factors in resistance of pea to the pea aphid, *Acyrthosiphon pisum* (Harr.) (Homoptera: Aphididae). 11. Amino acids. Canad. Entomol. 89: 457–464.

Banks, C. J. 1965. Aphid nutrition and reproduction. Rep. Rothamsted Exp. Stn. 1964: 299–309.

——— & E. D. M. Macaulay. 1964. The feeding, growth and reproduction of *Aphis fabae* Scop. on *Vicia faba* under experimental conditions. Ann. Appl. Biol. 53: 229–242.

——— & H. L. Nixon. 1958. Effects of the ant, *Lasius niger* L., on the feeding and excretion of the bean aphid, *Aphis fabae* Scop. J. Exptl. Biol. 35: 703–711.

Basden, R. 1968. The occurrence and composition of the sugars in the honeydew of *Eriococcus coriaceus* (Mask). Proc. Linn. Soc. New South Wales 92: 222–226.

Baudet, J. C. 1974. Signification taxonomiques des caracteres blastogeniques dans la tribu des Papillionaceae–Phaseoleae. Bull. Jard. Bot. Nat. Belg. 44: 259–293.

Berlinski, K. 1965. Badania nad pobieranien pokarmu i wplywem roslin zywicielskich na mszyce trzmielinowo-burakowa-*Aphis fabae* Scop. (Homopt., Aphididae). (Studies on food intake and the effects of food plants on the bean aphid-*Aphis fabae*.) Polskie Pismo Ent. (B) 163–168.

Bernard, R. L. & B. B. Singh. 1969. Inheritance of pubescence type in soyabeans; glabrous, curly, dense, sparse, puberulent. Crop Sci. 9: 192–197.

Birch, N. 1985. Field evaluation of resistance to black bean aphid, *Aphis fabae*, on close relatives of the faba bean, *Vicia faba*. Ann. Appl. Biol. 106: 561–569.

——— & S. D. Wratten. 1984. Patterns of aphid resistance in the genus *Vicia*. Ann. Appl. Biol. 104: 327–338.

Bond, D. A. & H. J. B. Lowe. 1975. Tests for resistance to *Aphis fabae* in field beans (*Vicia faba*). Ann. Appl. Biol. 81: 21–32.

Bradley, R. H. E. 1954. Studies of the mechanism of transmission of potato virus Y by the green peach aphid *Myzus persicae* (Sulz.) (Homoptera: Aphidae). Canad. J. Zool. 32: 64–73.

Bromley, A. K. & M. Anderson. 1982. An electrophysiological study of olfaction in the aphid *Nasonovia ribis-nigri*. Entomol. Exp. Appl. 32: 101–110.

———, J. A. Dunn & M. Anderson. 1979. Ultrastructure of the antennal sensilla of aphids 1. Coeloconic and placoid sensilla. Cell Tiss. Res. 203: 427–442.

Busgen, M. 1981. Der Honigtau. Eine biologische Studien an Pflanzen und Pflanzenlausen. Z. Naturwiss. (Jena) 25: 339–428.

Campbell, B. C., K. C. Jones & D. L. Dreyer. 1986. Discriminative behavioural responses by aphids to various plant matrix polysaccharides. Entomol. Exp. Appl. 41: 17–24.

Cockbain, A. J. 1961. Fuel utilization and duration of tethered flight in *Aphis fabae*. J. Exptl. Biol. 38: 163–174.

Corcuera, L. J. 1984. Effects of indole alkaloids from Gramineae on aphids. Phytochemistry 23: 539–541.

Cowan, R. S. 1981. Caesalpinioideae. Pp. 57–64 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legumes Systematics, Part 1. Royal Botanic Gardens, Kew.

Davidson, J. 1923. Biological studies of *Aphis rumicis*. L. The penetration of plant tissues and the source of the food supply of aphids. Ann. Appl. Biol. 10: 35–54.

Dixon, A. F. G. 1971. The life-cycle and host preferences of the bird cherry-oat aphid *Rhopalosiphon padi* (L.) and their bearing on the theories of host alteration in aphids. Ann. Appl. Biol. 68: 135–147.

———. 1973. The biology of aphids. Studies in Biology, Volume 44. Edward Arnold, London.

———. 1976. Reproductive strategies of the alate morphs of the bird-cherry aphid, *Rhopalosiphum padi* (L.). J. Anim. Ecol. 45: 817–830.

———. 1985. Aphid Ecology. Blackie, London.

——— & T. D. Dharma. 1980. Spreading the risk in developmental mortality: size, fecundity and reproductive rate in the black bean aphid. Entomol. Exp. Appl. 28: 301–312.

Dreyer, D. L. & K. L. Jones. 1981. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminus* and *Myzus persicae:* aphid feeding deterrents in wheat. Phytochemistry 20: 2489–2493.

———, K. C. Jones & R. J. Molyneux. 1985. Feeding deterrency of some pyrrolizidine, indolizidine, and quinolizidine alkaloids towards pea aphid *(Acyrthosiphon pisum)* and evidence for phloem transport of indolizidine alkaloid swainsonine. J. Chem. Ecol. 8: 1045–1051.

Eastop, V. F. 1973. Deductions from the present day host plants of aphids and related insects. Pp. 157–178 *in* H. F. Van Emden (editor), Insect–Plant Relationships, Symposium. R. Entomol. Soc. London, Volume 6. Blackwell, Oxford.

Eharhardt, P. 1961. Zue Nahrungsaufnahme von *Megoura viciae* Buckt., einer phloem-saugenden Aphide (Homoptera, Rhynchota). Experientia 17: 461–462.

Elias, T. S. 1981. Mimosoideae. Pp. 143–151 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Gibson, R. W. & J. A. Pickett. 1983. Wild potato repels aphids by release of aphid alarm pheromone. Nature 302: 608–609.

Harrewijn, P. & J. P. W. Noordink. 1971. Taste perception of *Mysus persicae* in relation to food uptake and development processes. Entomol. Exp. Appl. 14: 413–419.

Hartnett, D. C. & F. A. Bazzaz. 1984. Leaf demography and plant-insect interactions: goldenrods and phloem feeding aphids. Amer. Nat. 124: 137–142.

Harris, K. F. 1977. An ingestion-egestion hypothesis of non-circulative virus transmission. Pp. 165–220 *in* K. F. Harris & K. Maramorosch (editors), Aphids as Virus Vectors. Academic Press, New York.

Holt, J. 1981. Antibiotic resistance to *Aphis fabae* cultivars. Tests of agrochemicals and cultivars 2. Ann. Appl. Biol. 97 (Suppl.): 66–67.

——— & N. Birch. 1984. Taxonomy, evolution and domestication of *Vicia* in relation to aphid resistance. Ann. Appl. Biol. 105: 547–556.

——— & S. D. Wratten. 1986. Components of resistance to *Aphis fabae* in faba bean cultivars. Entomol. Exp. Appl. 40: 35–40.

Howe, W. L. & O. F. Smith. 1957. Resistance to the spotted alfalfa aphid in Lahontan alfalfa. J. Econ. Entomol. 50: 320–324.

Hughes, R. D. & M. A. Bryce. 1984. Biological characterization of two biotypes of pea aphid, one susceptible and the other resistant to fungal pathogens coexisting on lucerne in Australia. Entomol. Exp. Appl. 36: 225–229.

Hussain, A., J. H. S. Forrest & A. F. G. Dixon. 1973. Changes in growth regulator content of radish seedlings, *Raphanus sativus*, infested with the aphid *Myzus persicae*. Ann. Appl. Biol. 75: 275–284.

Ibbotson, A. & J. S. Kennedy. 1959. Interactions between walking and probing in *Aphis fabae* Scop. J. Exptl. Biol. 36: 377–390.

Irwin, M. E. 1978. Pests of soybean in the USA and their control. Pp. 141–150 *in* S. R. Singh & H. F. Van Emden (editors), Pests of Grain Legumes: Ecology and Control. Academic Press, London.

Johnson, B. 1953. The injurious effects of the hooked epidermal hairs of French beans (*Phaseolus vulgaris* L.) on *Aphis craccivora* Koch. Bull. Entomol. Res. 44: 779–788.

_____. 1966. Wing polymorphism in aphids, iii. The influence of the host plant. Entomol. Exp. Appl. 9: 213–222.

Johnson, C. G. 1969. Migration and Dispersal of Insects by Flight. Methuen, London.

Jordens, D. & F. Klingauf. 1977. Der Einfluss von L. Dopa auf Ansiedlung und Entwicklung von *Aphis fabae* an synthetischer Diat. Med. Fac. Landouww. Rijklsuniv. Gent 42: 1411–1419.

Jordens-Rottger, D. 1979. The role of phenolic substances for host-selection behaviour of the black bean aphis, *Aphis fabae*. Entomol. Exp. Appl. 26: 49–54.

Kennedy, C. E. J. 1986. The role of leaf surfaces in oak aphid host selection. Pp. 344–345 *in* B. E. Juniper & T. R. E. Southwood (editors), Insects and the Plant Surface. Edward Arnold, London.

Kennedy, J. S. 1961. Migration and dispersal of insects by flight. Nature, Lond. 189: 785–791.

_____. 1965. Mechanisms of host plant selection. Ann. Appl. Biol. 56: 317–322.

_____. 1966. The balance between antagonistic induction and depression of flight activity in *Aphis fabae* Scopoli. J. Exptl. Biol. 45: 215–228.

_____. 1985. Displacement activities and post-inhibitory rebound. Anim. Behav. 33: 1375–1377.

_____ & I. H. M. Fosbrooke. 1973. The plant in the life of an aphid. Pp. 129–140 *in* H. F. Van Emden (editor), Insect-Plant Relationships. Blackwell, Oxford.

_____ & A. R. Ludlow. 1974. Co-ordination of two kinds of flight activity in an aphid. J. Exptl. Biol. 61: 173–196.

Khamala, C. P. M. 1978. Pests of grain legumes and their control in Kenya. Pp. 127–137 *in* S. R. Singh, H. F. Van Emden & T. A. Taylor (editors), Pests of Grain Legumes: Ecology and Control. Academic Press, London.

Kidd, N. A. C. & A. M. Cleaver. 1984. The relationship between pre-flight reproduction and migratory urge in alatae of *Aphis fabae* Scopoli (Hemiptera: Aphididae). Bull. Entomol. Res. 74: 517–527.

Kimmins, F. M. 1986. Ultrastructure of the stylet pathway of *Brevicoryne brassicae* in host plant tissue, *Brassica oleracea*. Entomol. Exp. Appl. 41: 283–290.

Klingauf, F. 1972. Die Bedeutung von peripher vorliegenden Pfalanzen substanzen fur die Wirtswahl von phloemsaugenden Blattlausen (Aphididae). Z. Pflanzenkrankh. Pflanzenschutz. 79: 471–477.

_____. 1982. Breeding for resistance to aphids. Pp. 285–295 *in* G. Hawtin & C. Webb (editors), Faba Bean Improvement, Volume 6. World Crops: Production, Utilisation and Description. Martinus Nighoff.

Kloft, W. 1960. Die Trophobiose zwishen Waldameisen und Pflanzenlausen mit Untersuchungen uber die Wechselwirkungen zwischen Pflanzenlausen und Pflanzengeweben. Entomophaga 5: 43–54.

Kring, J. B. 1969. Behavioural responses of winged bean aphids to coloured fluorescent lamps. J. Econ. Entomol. 62: 1450–1455.

Lackey, J. A. 1978. Leaflet anatomy of Phaseoleae (Leguminosae, Papilionoideae) and its relation to taxonomy. Bot. Gaz. (Crawfordsville) 139: 436–446.

Lapointe, S. L. & W. M. Tingey. 1984. Feeding response of the green peach aphid (Homoptera: Aphididae) to potato glandular trichomes. J. Econ. Entomol. 77: 386–389.

Leckstein, P. M. & M. Llewellyn. 1973. The role of amino acids in diet intake and selection and the utilization of dipeptides by *Aphis fabae*. J. Insect. Physiol. 20: 877–885.

Lees, A. D. 1966. The control of polymorphism in aphids. Adv. Insect Physiol. 3: 207–277.

Levin, D. A. 1973. The role of trichomes in plant defense. Quart. Rev. Biol. 48: 3–15.

Llewellyn, M. & V. K. Brown. 1985. A general relationship between adult weight and the reproductive potential of aphids. J. Anim. Ecol. 54: 663–673.

_____ & P. M. Leckstein. 1978. A comparison of energy budgets and growth efficiency for *Aphis fabae* Scop. reared on synthetic diets with aphids reared on broad beans. Entomol. Exp. Appl. 23: 66–71.

_____ & A. L. Qureshi. 1978. The energetics and growth efficiency of *Aphis fabae* reared on different parts of the broad bean plant *(Vicia faba)*. Entomol. Exp. Appl. 23: 26–39.

_____ & _____.1979. The energetics of *Megoura viciae* reared on different parts of the broad bean plant *(Vicia faba)*. Entomol. Exp. Appl. 26: 127–135.

Lowe, H. J. B. 1967. Interspecific differences in the biology of aphids (Homoptera: Aphididae) on the leaves of *Vicia faba:* 1. Feeding behaviour. Entomol. Exp. Appl. 10: 347–357.

Mansour, M. H., N. Z. Dimetry & I. S. Rofaeel. 1982. The role of coumarin as secondary plant substance in the food specificity of the cowpea aphid *Aphis craccivora* Koch. Z. Angew. Entomol. 93: 151–157.

Marek, J. 1961. Die Wirkung von Aphidenstichen auf Pflanzliche Zellen. Entomol. Exp. Appl. 4: 20–34.

McAllen, J. W. & J. B. Adams. 1961. The significance of pectinase in plant penetration by aphids. Canad. J. Zool. 39: 305–310.

McLean, D. L. & M. G. Kinsey. 1968. Probing behaviour of the pea aphid, *Acyrthosiphon pisum.* ii. Comparisons of salivation and ingestion in host and non-host leaves. Ann. Entomol. Soc. Amer. 61: 730–739.

_____ & _____. 1984. The precibarial valve and its role in feeding behaviour of the pea aphid, *Acyrthosiphon pisum.* Bull. Entomol. Soc. Amer. 30: 26–31.

Miles, P. W. 1964. Studies on the salivary physiology of plant bugs: the chemistry of formation of the sheath material. J. Insect Physiol. 10: 147–160.

Mittler, T. E. 1970. Uptake rates of plant sap and synthetic diet by the aphid *Myzus persicae.* Ann. Entomol. Soc. Amer. 63: 1701–1705.

_____. 1971. Some effects on the aphid *Myzus persicae* of ingesting antibiotics incorporated into artificial diets. J. Insect Physiol. 17: 1333–1374.

Müller, F. P. 1962. Biotypen und Unterarten der "Erbenblattlaus" *Acyrthosiphon pisum* (Harris). Z. Pflanzenkrankh., Pflanzenpathol. Pflanzenschutz 69: 130–136.

_____. 1971. Isolationsmechanismen zwischen sympatrischen bionomischen Rassen am Beispiel der Erbenblattlaus *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae). Zool. Jb. Syst. 98: 131–152.

_____. 1980. Wirtspflanzen, Generationenfolge und reproduktive Isolation infraspezifischer Formen von *Acyrthosiphon pisum.* Entomol. Exp. Appl. 28: 145–157.

Müller, H. J. 1958. The behaviour of *Aphis fabae* in selection of its host especially different varieties of *Vicia faba.* Entomol. Exp. Appl. 1: 181–190.

_____. 1961. Über die Ursachen der unterschiedlichen Resistenz von *Vicia faba* L. gegenüber der Bohnenblattlaus. *Aphis (Doralis) fabae* Scop. VII. Reproduktionsrate und Korpengrosse von *Aphis fabae* auf gleichaltrigen Jungpflanzen unterschiedlichen Wuchsigkeil. Entomol. Exp. Appl. 4: 148–164.

Nault, L. R. & G. G. Gyrisco. 1966. Relation of the feeding process of the pea aphid to the innoculation of pea enation mosaic virus. Ann. Entomol. Soc. Amer. 59: 1185–1197.

Nyiira, Z. M. 1978. Pests of grain legumes and their control in Uganda. Pp. 117–121 *in* S. R. Singh & H. F. Van Emden (editors), Pests of Grain Legumes: Ecology and Control. Academic Press, London.

Parry, W. H. 1977. The effects of nutrition and density on the production of alate *Elatobium abietinium* on Sitka Spruce. Oeceologia 30: 367–375.

Phelan, P. L. & J. R. Miller. 1982. Postlanding behaviour of *Myzus persicae* as altered by (E)-β-farnesene and three carboylic acids. Entomol. Exp. Appl. 32: 46–53.

Poehling, H. M. & Y. Morvan. 1984. Untersuchungen zur Entwicklung von *Aphis fabae* an restenten und anfalligen Sorten von *Vicia faba.* Med. Fac. Landbouww. Rijksuniv. Gent 49: 793–802.

Polhill, R. M. 1981. Papilionoideae. Pp. 191–208 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bull. Entomol. Res. 62: 631–714.

_____. 1977. Aphid penetration of plant tissues. Pp. 105–118 *in* K. F. Harris & K. Maramorosch (editors), Aphids as Virus Vectors. Academic Press, New York.

Poos, F. W. 1929. Leafhopper injury to legumes. J. Econ. Entomol. 22: 146–153.

_____ & F. F. Smith. 1931. A comparison of oviposition and nymphal development of *Empoasca fabae* (Harris) on different host plant. J. Econ. Entomol. 84: 361–371.

Qin Junde & Ke Lidao. 1984. The influence of secondary plant substances on the growth and development of *Myzus persicae* of Beijing. Entomol. Exp. Appl. 35: 17–20.

Raccah, B. & A. S. Tahari. 1971. Wing dimorphism in influencing resistance or toxicity tests and food uptake in *Myzus persicae.* Entomol. Exp. Appl. 14: 310–314.

Salyk, R. P. & D. J. Sullivan. 1982. Comparative feeding behaviour of two aphid species: bean aphid (*Aphis fabae* Scopoli) and pea aphid (*Acyrthosiphon pisum* (Harris)). J. New York Entomol. Soc. XC: 87–93.

Scheurer, S. 1976. The influence of phytohormones and growth regulatory substances on insect developmental processes. Sym. Biol. Hung. 16: 255–259.

Schoonhoven, L. M. & J. Derken-Koppers. 1976. Effects of some allelochemics on food uptake and survival of a polyphagous aphid, *Myzus persicae* Sulz. Entomol. Exp. Appl. 19: 52–56.

Shambaugh, G. F., J. L. Frazier, A. E. H. Castell & L. B. Coons. 1978. Antennal sensilla of 17 aphid species (Homoptera: Aphidinae). Int. J. Insect Morphol. & Embryol. 7: 389–404.

Shaw, M. J. P. 1970. Effects of population density on alienicolae of *Aphis fabae* Scop. 11. The effects of crowding on the migratory urge among aphids in the laboratory. Ann. Appl. Biol. 65: 197–203.

Smith, B. D. 1966. Effect of plant alkaloid sparteine on the distribution of the aphid *Acyrthosiphon spartii* (Koch). Nature 212: 213–214.

Sorin, M. 1962. The life history of *Melanaphis bambusae* (Fullaway), and the penetration of stylets into host plants. Kontyu 30: 222–229.

_____. 1966. Physiological and morphological studies on the suction mechanism of plant juice by aphids. Bull. Univ. Osaka Prefect (B) 18: 95–137.

Srivastava, P. N. & J. L. Auclair. 1962. Characteristics of invertase in the alimentary canal of the pea aphid, *Acyrthosiphon pisum* (Harr). J. Insect Physiol. 8: 527–535.

_____, J. L. Auclair & U. Srivastava. 1983. Effect of nonessential amino acids on phagostimulation and maintenance of the pea aphid, *Acyrthosiphon pisum*. Canad. J. Zool. 61: 2224–2229.

_____, Y. Gao, J. Levesque & J. L. Auclair. 1985. Differences in amino acid requirements between two biotypes of the pea aphid, *Acyrthosiphon pisum*. Canad. J. Zool. 63: 603–606.

Sutherland, O. R. W. 1967. Role of host plant in production of winged forms by a green strain of pea aphid, *Acyrthosiphon pisum* (Harris). Nature 216: 387–388.

_____. 1969. The role of the host plant in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. J. Insect. Physiol. 15: 2170–2201.

Tarn, T. R. & J. B. Adams. 1982. Aphid probing and feeding, electronic monitoring and plant breeding. Pp. 221–246 *in* K. F. Harris & K. Maramorosch (editors), Pathogens, Vectors and Plant Diseases: Approach to Control. Academic Press, New York.

Tjallingii, W. F. 1978. Mechanoreceptors of the aphid labium. Entomol. Exp. Appl. 24: 531–537.

_____. 1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. Entomol. Exp. Appl. 38: 187–193.

Van Emden, H. F. 1969. Induced by Chemicals. J. Sci. Fd. Agric. 20.

_____. 1972. Aphids as Phytochemists. Pp. 25–43 *in* J. B. Harborne (editor), Phytochemical Ecology. Academic Press, London.

Visser, J. H. & J. W. Taanman. 1987. Odour-conditioned anemotaxis of apterous aphids *(Cryptomyzus korschelti)* in response to host plants. Physiol. Entomol. 12: 473–479.

Watson, M. A. & R. T. Plump. 1972. Transmission of plant-pathogenic viruses by aphids. Ann. Rev. Entomol. 17: 425–452.

Wensler, R. J. D. & B. K. Filshie. 1969. Gustatory sense organs in the food canal of aphids. J. Morphol. 129: 473–492.

Winks, M., T. Hartmann, L. Witte & J. Reinheimer. 1982. Inter-relationship between quinolizidine alkaloid producing legumes and infesting insects: exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cystisorum*. Z. Naturforsch. 37c: 1081–1086.

Wright, J. P. & D. B. Fisher. 1980. Direct measurement of sieve tube turgor pressure using severed aphid stylets. Pl. Physiol. 65: 1133–1135.

Zettler, F. W., M. C. Smyly & I. R. Evans. 1969. Effect of probing behaviour and starvation of *Myzus persicae* on transmission of bean common mosaic virus. Phytopathology 56: 1079–1082.

Zindler-Frank, E. 1987. Calcium-oxalate crystals in legumes. *In:* C. H. Stirton (editor), Advances in Legume Systematics, 3. Royal Botanic Gardens, Kew (in press).

ADAPTIVE RADIATION OF *ACANTHOSCELIDES* IN SEEDS: EXAMPLES OF LEGUME-BRUCHID INTERACTIONS

Clarence Dan Johnson[1]

Abstract

Larvae of seed beetles (Bruchidae) feed and live in the seeds of about 33 families of plants, but over 80% eat seeds in the Leguminosae. Other families that are fed upon most frequently are the Convolvulaceae, Palmae, Malvaceae, Combretaceae, Rhamnaceae, and Tiliaceae. As a whole, many species of seeds of wild plants are eaten, especially in the tropics. Most bruchids complete only one or a few generations per year; however, the majority of those that are of economic importance continue to feed in seeds until the seeds are used up. Most bruchids are not economically important, although they may become more economically important if noneconomic plants are developed for economic purposes. Bruchids that feed in several species or genera of plants have a wider geographic distribution than those that feed in only one or a few hosts. Ecological conditions such as destruction of natural habitats, which limits the number of host plants, also may influence the distribution of bruchids. Secondary compounds (toxins) in seeds prevent the attacks of most bruchids and probably account for their high host specificity. Most (80%+) species of bruchids are specialists, feeding on one to three species of plants. Many bruchids are specific to toxic seeds, and some even utilize the toxins for their dietary needs. Some 31 defensive traits of seeds and pods against bruchids have been suggested, and at least 11 of these have been overcome by bruchids in the process of coevolution or evolution. Of these, toxic seeds and predator satiation seem to be the most important. At least three feeding guilds of bruchids are known. Some lay eggs on mature pods, others on mature seeds on the plants, and others lay eggs on seeds after they have fallen to the substrate (have been dispersed). Thus the seeds of some plants may be attacked at all stages of development, once the seed is large enough to be eaten or lived in, and even after dispersal. *Acanthoscelides* is the largest genus of New World Bruchidae, with about 250 species described and as many yet to be described. Adaptive radiation of species of *Acanthoscelides*, as well as all bruchids, has taken place primarily among papilionoid and mimosoid legumes, and the Malvaceae. Except for *Cassia* (s.l.), few feed in caesalpinioid legumes. Species groups of *Acanthoscelides* that are formed on the basis of their morphology, generally feed in the same subfamily of legumes or family of plants. Species grouped in this way often will have one species that has evolved to feed in the seeds of a different family. The genera *Mimosa, Astragalus, Desmodium, Dalea, Rhynchosia, Leucaena, Desmanthus,* and *Parkia* are used most frequently as hosts by *Acanthoscelides*. Based on ratios of species fed upon by bruchids and number of species in a taxon, the Mimosoideae are fed upon more frequently than any other subfamily (ratio 1:30). The Malvaceae have a ratio of 1:31, the Papilionoideae 1:71, the Caesalpinioideae 1:192, and the Leguminosae 1:64. The Leguminosae are fed upon by many species in other genera of Bruchidae, making it the most frequently used host of the seed beetles.

I. Introduction

Beetles in the family Bruchidae feed in the seeds of about 33 families of plants, although most feed in the Leguminosae. The reasons for host specificity to any family and especially to the Leguminosae are not ascertained readily, but some possible reasons are discussed below.

Bruchids are in the superfamily Chrysomeloidea, which includes leaf beetles and longhorned wood borers. The larvae of leaf beetles feed in or mine the leaves, roots, and stems. The larvae of longhorned wood borers feed in wood and roots. All known seed beetles feed on and usually live in seeds.

[1]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A.

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The family Bruchidae consists of about 1,300 described species and about 56 genera grouped in the subfamilies Amblycerinae, Bruchinae, Eubaptinae, Kytorhininae, Pachymerinae, and Rhaebinae (Southgate, 1979). About 80% of bruchid species are in the Bruchinae, 10% in the Amblycerinae, 9% in the Pachymerinae, and 1% assigned to the other three subfamilies.

The life histories of bruchids vary considerably in oviposition, larval entry, and larval feeding behavior. The general life cycle is that the adult lays an egg on a pod or seed, and the first-stage (instar) larva bores through the pod and/or seed coat and enters the seed. The first-stage larva is highly modified to enter seeds and has many spines and often well-developed legs. Once it has entered a seed it molts into a legless grub and commences to feed. A larva usually molts three more times as it continues to feed inside the seed; usually only one, but sometimes several seeds are eaten. Bruchids usually pupate inside the seed that the larva has fed upon, but those that feed on several seeds may build a pupal chamber by gluing several seeds together, or some may leave the seeds and spin a cocoon in which they pupate. The larva starts and usually the adult finishes making a round exit hole in the seed and perhaps in the pod through which the adult exits. Adult bruchids feed on pollen and nectar and are not known to feed on seeds or pods. Some species of bruchids may complete many generations in a year (homodynamic life cycles), but most species of bruchids complete only one or a few generations per year (heterodynamic life cycles). In the laboratory or in stored seeds, bruchids may complete many generations without the adults feeding; however, it has been shown that adult bruchids will lay more eggs if provided with ample food and water, and they are suspected to feed on flowers, pollen, and nectar in the field.

According to Johnson (1981a) it is likely that the chrysomeloid ancestors of bruchids radiated onto the seeds of the Leguminosae, or possibly its ancestors, shortly after it arose and later evolved the ability to feed in seeds of other families.

About 84% of the hosts of bruchids are in the Leguminosae (Johnson, 1970). Most of the other hosts are in the families Convolvulaceae (4.5%), Palmae (4.5%), and Malvaceae (2%). The remaining 5% are in 29 other families. Of the 5%, the Combretaceae, Rhamnaceae, Sterculiaceae, and especially the Tiliaceae host more bruchids than the other families. Johnson (1981a) listed host families and discussed bruchid host preferences. He also stressed that many more hosts for bruchids remain to be found, especially in tropical America, Africa, Asia, and possibly much of Australia. He also found in an analysis of host preferences of bruchids in the legumes that bruchids are primarily specific to taxa of legumes from the generic to the family level, but are usually not specific to particular species of hosts over the entire range of the bruchids.

Johnson (1981a) discussed the distribution, ecology, and economic importance of bruchids. He pointed out that bruchids and their relationships with hosts make them especially good organisms for studies of plant–animal interactions, and he briefly discussed these interactions as they relate to coevolution.

II. The Genus *Acanthoscelides*

Acanthoscelides is the largest genus of New World Bruchidae, with about 250 species and perhaps as many new species that are not yet described. The genus is a natural taxon that has many characteristics which ally it to other New World genera; when a systematist finds a species that is not readily placed in a genus, it is often found to be an *Acanthoscelides*.

In general, species that feed on several species or genera of plants have a wider distribution than those that feed in only one host (Johnson & Kingsolver, 1973; Johnson & Slobodchikoff, 1979). *A. obtectus* is an extreme example because of the economic importance of its hosts and their cosmopolitan distribution. Presumably because seeds of native hosts of *A. obtectus* have been placed in storehouses along with nonnative hosts, *A. obtectus* has had the opportunity to adapt to feeding in seeds of more than 18 species of economic importance. This phenomenon may be observed in other, noneconomic species of bruchids if the bruchids are stored under similar circumstances (e.g., *Stator*

pruininus (Horn) readily feeds in seeds of lentils in the laboratory). A more typical example of a bruchid with a wide distribution is *A. pertinax;* it feeds on seeds of four genera of plants and has a distribution from northern Mexico to Venezuela. Another species, *A. puellus*, seems to be an exception because it has a distribution from northern Mexico to Panama but has only one known host. The host, *Calopogonium mucunoides*, has a broad range and *A. puellus* occurs throughout much of that range. More species of *Acanthoscelides* feed on papilionoid legumes than on any other taxon (Table 1).

TABLE 1. *Acanthoscelides* species and their host plants. The *Acanthoscelides* Species Group number is in parentheses following each species name.

Acanthoscelides species	Host plants
aequalis (12)	*Abutilon californicum*
	A. incanum
	A. trisulcatum
	Pseudabutilon lozani
	Wissadula amplissima
	W. holoserica
alboscutellatus (5)	*Ludwigia alternifolia*
	L. palustris
amabilis (4)	*Rhynchosia discolor*
ambopygus (?)	*Apuleia leiocarpa*
anoditus (12)	*Anoda cristata*
apicalis (12)	*Malachra* spp.
argillaceus (1)	*Dolichos lablab*
	Phaseolus lunatus
argutus (7)	*Teramnus uncinatus*
	T. volubilis
atomus (4)	*Lechea tenuifolia*
atronotatus (?)	*Lithraea brasiliensis*
aureolus (4)	*Astragalus allochrous*
	A. amphioxys
	A. antiselli
	A. asymmetricus
	A. bisulcatus
	A. calycosus
	A. crotalariae
	A. douglasii
	A. drummondii
	A. fucatus
	A. grayii
	A. humistratus
	A. lancearius
	A. lentiginosus
	A. mollissimus
	A. oxyphysus
	A. pattersoni
	A. praelongus
	A. racemosus
	A. wootoni
	Glycyrrhiza lepidota
	Lotus purshianus
	L. scoparius

TABLE 1. Continued.

Acanthoscelides species	Host plants
	Oxytropis lambertii
	O. sericea
aurulentus (9)	*Parkia cachimboensis*
	P. decussata
	P. discolor
	P. igneifolia
	P. nitida
	P. panurensis
	P. ulei
baboquivari (5)	*Indigofera platycarpa*
	I. sphaerocarpa
bellus (11)	*Triumfetta lappula*
	T. paniculata
bilobatus (9)	*Apuleia leiocarpa*
bisagittus (9)	*Parkia discolor*
	P. igneifolia
	P. panurensis
bisignatus (9)	*Desmanthus illinoensis*
	D. leptolobus
	D. velutinus
biustulus (7)	*Desmodium batocaulon*
	D. cinerascens
	D. grahamii
	D. hartwegianum
	D. neomexicanum
	D. nitidum
blanchardi (6)	*Kosteletzkya tubiflora*
boneti (9)	*Leucaena diversifolia*
	L. esculenta
brevipes (12)	*Malvastrum americanum*
	M. bicuspidatum
	M. corchorifolium
	Sida acuta
	S. aggregata
	S. rhombifolia
	S. spinosa
calvus (4)	*Helianthemum canadense*
campeche (5)	*Rhynchosia longeracemosa*
	R. reticulata
caracallae (4)	*Phaseolus caracalla*
	Vigna sp.
chiricahuae (10)	*Mimosa aculeaticarpa*
	M. albida
	M. arida
	M. biuncifera
	M. borealis
	M. brandegei
	M. depauperata
	M. dysocarpa
	M. emoryana
	M. grahamii

TABLE 1. Continued.

Acanthoscelides species	Host plants
	M. lacerata
	M. laxiflora
	M. monancistra
	M. palmeri
clandestinus (4)	*Phaseolus vulgaris*
clitellarius (9)	*Piptadenia flava*
compressicornis (5)	*Desmanthus acuminata*
	D. cooleyi
	D. covillei
	D. depressus
	D. illinoensis
	D. leptolobus
	D. obtusus
	D. subulatus
	D. velutinus
	D. virgatus
	Hoffmanseggia densiflora
	H. drepanocarpa
	H. tenella
	Phaseolus aff. *peduncularis*
comptus (4)	*Mimosa albida*
cordifer (9)	*M. guanacastensis*
	M. langlassei
	M. velloziana
	Heliocarpus palmeri
cornis (11)	*Desmodium distortum*
cuernavaca (7)	*D. orbiculare*
	D. tortuosum
	Dalea fremontii
daleae (4)	*D. schottii*
	D. spinosa
	Desmanthus covillei
derifieldi (7)	*D. subulatus*
	Desmanthus covillei
desmanthi (9)	*D. virgatus*
	D. virgatus var. *depressus*
	Desmodium tortuosum
desmodicola (7)	*Desmodium amplifolium*
desmoditus (7)	*D. angustifolium*
	D. cinereum
	D. distortum
	D. nicaraguense
	D. orbicularis
	D. orbicularis var. *salvini*
	D. orbicularis var. *rubricaule*
	D. rensoni
	D. skinneri
	D. tortuosum
	Mimosa dormiens
devriesi (3)	*Mimosa albida*
difficilis (3)	*M. albida* var. *strigosa*

TABLE 1. Continued.

Acanthoscelides species	Host plants
	M. guanacastensis
	M. langlassei
	M. pigra
	M. pudica
	M. pusilla
	M. velloziana
distinguendus (5)	*Rhynchosia americana*
	R. difformis
	R. erecta
	R. galactioides
	R. intermedia
	R. latifolia
	R. tomentosa
elkinsae (12)	*Hibiscus* sp.
eriosemicola (1)	*Eriosema grandiflorum*
flavescens (5)	*Eriosema violaceum*
	Galactia striata
	Rhynchosia longeracemosa
	R. minima
	R. phaseoloides
floridae (4)	*Amorpha fruticosa*
fraterculus (4)	*Astragalus bisulcatus*
	A. caryocarpus
	A. cibarius
	A. crassicarpus
	A. drummondii
	A. hyalinus
	A. missouriensis
	A. mollissimus
	A. pattersoni
	A. pectinatus
	A. racemosus
	A. utahensis
	Glycyrrhiza lepidota
	Lotus crassifolius
	L. mearnsii
	L. rigidus
fryxelli (6)	*Kosteletzkya depressa*
	K. hispidula
	Malachra capitata
	Malachra sp.
fumatus (4)	*Rhynchosia texana*
griseolus (4)	*Calopogonium mucunoides*
	Sesbania emerus
	S. exaltata
	S. macrocarpa
guazumae (12)	*Guazuma ulmifolia*
guerrero (12)	*Herissantia crispa*
	Malvastrum americanum
hectori (5)	*Calopogonium caeruleum*
	Macroptilium atropurpureum
	Rhynchosia minima

TABLE 1. Continued.

Acanthoscelides species	Host plants
helenae (9)	*Parkia decussata*
helianthemum (4)	*Helianthemum corymbosum*
herissantitus (12)	*Herissantia crispa*
	Malvastrum bicuspidatum
hibiscicola (6)	*Hibiscus* sp.
hopkinsi (9)	*Parkia decussata*
	P. discolor
	P. igneifolia
	P. multijuga
	P. nitida
	P. panurensis
	P. ulei
	P. velutina
howdenorum (7)	*Desmodium cinereum*
imitator (9)	*Parkia pendula*
	P. platycephala
indigoferestes (4)	*Indigofera suffruticosa*
isla (5)	*Rhynchosia calycosa*
johni (12)	*Herissantia crispa*
johnsoni (11)	*Heliocarpus palmeri*
	H. popayanensis
	Triumfetta calderoni
	T. lappula
kingsolveri (4)	*Indigofera costaricensis*
	I. densiflora
	I. lindheimeriana
	I. panamensis
	I. platycarpa
	I. sphaerocarpa
	I. suffruticosa
lambda (9)	*Parkia decussata*
	P. multijuga
lapsanae (9)	*Mimosa panamensis*
	M. pudica
leucaenicola (9)	*Leucaena diversifolia*
	L. esculenta
	L. lanceolata
lobatus (4)	*Astragalus humistratus*
	A. lentiginosus
	A. mollissimus
	A. mollissimus var. *bigelovii*
	A. mollissimus var. *marcidus*
	A. troglodytus
	Prosopis strombulifera
longescutus (?)	*Lespedeza capitata*
longistilus (7)	*L. hirta*
	L. texana
	L. virginica
	Leucaena collinsii
macrophthalmus (9)	*L. esculenta*
	L. glauca

TABLE 1. Continued.

Acanthoscelides species	Host plants
	L. guatemalensis
	L. lanceolata
	L. leucocephala
	L. macrophylla
	L. pulverulenta
	L. retusa
	L. salvadorensis
	L. shannoni
malvastrumicis (12)	*Malvastrum americanum*
malvitus (12)	*Abutilon* sp.
	Malva sp.
manducus (11)	*Triumfetta bogotensis*
mankinsi (9)	*Leucaena collinsii*
	L. esculenta
	L. glauca
	L. lanceolata
	L. leucocephala
	L. macrophylla
	L. shannoni
margaretae (4)	*Astragalus pycnostachyus*
mazatlan (7)	*Desmodium cinereum*
	D. distortum
	D. glabrum
	D. scorpiurus
	D. tortuosum
megacornis (11)	*Aeschynomene americana*
	Desmodium cinereum
	D. tortuosum
merida (12)	*Abutilon umbellatum*
mexicanus (9)	*Mimosa* nr. *albida*
	M. biuncifera
	M. brandegei
	M. egregia
	M. eurycarpa
	M. eurycarpoides
	M. galeottii
	M. lacerata
	M. laxiflora
	M. monancistra
	M. palmeri
	M. platycarpa
	M. polyanthoides
	M. setigera
mimosicola (3)	*Mimosa albida*
	M. langlassei
	M. pudica
mixtus (4)	*Astragalus allochrous*
	A. lentiginosus
	A. praelongus
	A. praelongus var. *lonchopus*
	A. sabulonum

TABLE 1. Continued.

Acanthoscelides species	Host plants
	A. thurberi
	A. wootoni
modestus (11)	*Aeschynomene ciliata*
	A. rudis
	A. scabra
	A. sensitiva
mundulus (14)	*Chaetocalyx latisiliqua*
	Nissolia fruticosa
	N. schottii
nigronotaticeps (?)	*Mimosa farinosa*
	M. detinens
oblongoguttatus (13)	*Acacia cornigera*
	A. cornigera + *A. sphaerocephala*
	A. sphaerocephala
obrienorum (9)	*Senna armata*
	S. atomaria
	S. biflora
	S. pallida
	S. pallida var. *gaumeri*
	S. polyantha
	S. racemosa
	S. skinneri
	S. villosa
	S. wislizenii
obsoletus (5)	*Tephrosia ambigua*
	T. spicata
	T. virginiana
obtectus (1)	*Cajanus indicus*
	Cicer arietinum
	Lathyrus sativus
	Lens esculenta
	Phaseolus aconitifolius
	P. acutifolius
	P. acutifolius var. *latifolius*
	P. aureus
	P. calcaratus
	P. caracalla
	P. coccineus
	P. lunatus
	P. vulgaris
	Pisum sativum
	Sesbania aegyptiaca
	Vicia faba
	Vigna sesquipedalis
	V. unguiculata
	Phaseolus glabellus
obvelatus (1)	*P. vulgaris*
	Petalostemum ornatus
oregonensis (4)	*Kosteletzkya depressa*
orlandi (6)	*Malachra capitata*
	Malachra sp.

TABLE 1. Continued.

Acanthoscelides species	Host plants
pallidipennis (4)	*Amorpha californica*
	A. canescens
	A. fruticosa
	Errazurizia rotundata
	Parryella filifolia
palmasola (4)	*Rhynchosia longeracemosa*
pauperculus (7)	*Trifolium obtusiflorum*
pavoniestes (6)	*Pavonia candida*
pectoralis (3)	*Desmanthus obtusus*
	D. velutinus
	D. virgatus
pedicularius (7)	*Petalostemum* sp.
perforatus (4)	*Astragalus canadensis*
pertinax (7)	*Aeschynomene americana*
	Dalea aff. *submontana*
	D. leporina
	Desmodium tortuosum
	Stylosanthes sp. prob. *humilis*
petalopygus (8)	*Acacia collinsii*
phalaropus (9)	*Parkia decussata*
	P. discolor
	P. nitida
	P. panurensis
	P. ulei
piceoapicalis (9)	*Mimosa casta*
	M. invisa
	M. pudica
	M. quadrivalvis
	M. somnians
pigricola (3)	*Mimosa pigra*
prosopoides (4)	*Ziziphus obtusifolia*
puelliopsis (7)	*Desmodium adscendens*
	D. barbatum
	D. incanum
	D. intortum var. *intortum*
puellus (4)	*Calopogonium mucunoides*
pullus (4)	*Astragalus allochrous*
	A. antiselli
	A. asymmetricus
	A. bolanderi
	A. crotalariae
	A. douglasii
	A. lentiginosus
	A. oxyphysus
	A. praelongus
	A. thurberi
	A. trichopodus
	A. wootoni
puniceus (3)	*Mimosa dormiens*
	M. pigra

TABLE 1. Continued.

Acanthoscelides species	Host plants
pusillimus (2)	*Dalea bicolor* var. *naviculifolia*
	D. carthagenensis
	D. cliffortiana
	D. leporina
	D. leucostachys var. *eysenhardtioides*
	D. scandens
	D. scandens var. *paucifolia*
	D. aff. *submontana*
	D. tomentosa
	Dalea zimapanica
	Trifolium pratense
	Sida pyramidata
pyramididos (12)	*Trifolium pratense*
pyrrhomelas (?)	*Mimosa pigra*
quadridentatus (3)	*M. pigra* var. *berlandieri*
	M. strigillosa
	Rhynchosia phaseoloides
rhynchosiestes (4)	*R. pyramidalis*
	Indigofera densiflora
ruficoxis (4)	*Galactia wrightii*
rufovittatus (4)	*Tephrosia cana*
	T. cinerea
	T. multifolia
	T. rhodantha
	T. tenella
	T. thurberi
	Heliocarpus attenuatus
sanblas (11)	*Triumfetta calderoni*
	T. lappula
	T. semitriloba
	T. speciosa
	Pachyrhizus erosus
sanfordi (4)	*Rhynchosia* aff. *yucatanensis*
	Mimosa near *albida*
schrankiae (10)	*M. bahamensis*
	M. eurycarpa
	M. eurycarpoides
	M. hemiendyta
	M. polyanthoides
	M. quadrivalvis
	M. setigera
	Schrankia diffusa
	S. distachya
	S. microphylla
	S. paucijuga
	S. roemeriana
	S. uncinata
	Desmodium incanum
schubertae (7)	*Dalea aurea*
seminulum (4)	*D. enneandra*

TABLE 1. Continued.

Acanthoscelides species	Host plants
	D. frutescens
	Petalostemum candidum
	P. feayi
	P. stanfieldii
sleeperi (12)	*Abutilon andrieuxii*
	A. palmeri
sousai (8)	*Acacia cornigera*
	A. gentlei
speciosus (9)	*Mimosa biuncifera*
	M. malacophylla
stylifer (7)	*Desmodium grahami*
suaveolus (4)	*Vigna peduncularis*
	V. vexillata
subaequalis (12)	*Abutilon berlandieri*
	A. californicum
	A. incanum
	A. lignosum
	A. pringlei
	A. trisulcatum
submuticus (4)	*Amorpha californica*
	A. fruticosa
	A. fruticosa var. *angustifolia*
	A. fruticosa var. *occidentalis*
surrufus (4)	*Rhynchosia reticulata*
taboga (4)	*Calopogonium caeruleum*
	C. mucunoides
	Pachyrhizus erosus
tenuis (4)	*Lythrum alatum*
	L. lineare
tepic (12)	*Abutilon amplexifolium*
tridenticulatus (3)	*Mimosa somnians*
	M. strigillosa
triumfettae (3)	*Triumfetta calderoni*
	T. lappula
unguiculatus (?)	*Apuleia leiocarpa*
zebratus (3)	*Mimosa pigra*
zeteki (5)	*Cajanus bicolor*
	C. cajan
	Flemingia strobilifera
	Rhynchosia calycosa
	R. longeracemosa
	R. minima
	R. pyramidilis
	R. reticulata
zonensis (7)	*Teramnus labialis*
	T. volubilis

III. Plant Defenses

TOXINS

Seeds often contain chemicals that are toxic to most organisms; it is therefore possible that toxins in seeds account for much of the host specificity of bruchid beetles. Janzen (1969) found that in the tropical lowlands of Costa Rica, large seeds were toxic and presumably protected from bruchids, and smaller seeds were less toxic and fed upon by bruchids. Seed fitness is apparently determined by seed size. Thus, individual large seeds have a better chance of survival than many small seeds. If a plant invests energy in large, toxic seeds, when germination occurs a seedling is produced that has a much better chance to survive. On the other hand, small seeds have less stored energy and toxins, are dispersed in great quantities, and after they germinate have less chance to develop into viable seedlings.

There is no doubt that seeds of legumes contain toxins. That these toxins protect seeds from predation by animals is not doubted. What is of interest is that bruchid beetles are able to feed in seeds that are very toxic. Janzen (1977), Johnson (1970), Center & Johnson (1974), Janzen et al. (1977), and Rosenthal & Bell (1979), among others, have presented examples of bruchids that feed in very toxic seeds. Johnson (1970) and Nelson & Johnson (1983b) presented evidence that several species of *Acanthoscelides* are able to feed in seeds of *Astragalus* that contain selenium. The latter authors found that some species are much more successful in feeding in selenium-containing seeds than are others. However, success in feeding in these seeds probably was due to the level of selenium in seeds. One species was able to tolerate very high levels of selenium while others could tolerate only much lower, but still toxic, levels. Center & Johnson (1974) found examples of bruchids that either are able to tolerate toxins or avoid them. The most intensive study on how bruchids are able to deal with toxins has been done in the laboratory of G. Rosenthal. The bruchid *Caryedes brasiliensis* feeds only in seeds of *Dioclea megacarpa*, which are rich in the toxic amino acid canavanine. This bruchid is able to detoxify canavanine in a manner that produces dietary nitrogen by converting canavanine to canaline and urea, and then degrading urea to ammonia (Rosenthal & Bell, 1979; Rosenthal & Janzen, 1983, 1985). Additional studies similar to those of Rosenthal et al. will undoubtedly unearth many mechanisms whereby bruchids are able to use toxic chemicals for their metabolism.

SEED SIZE

Janzen (1969) was the first to suggest that seed size and especially number had an effect on the process of predation by bruchids on seeds. He hypothesized that one of the reasons that plants produce many small seeds is that bruchids could not utilize all of them before they were dispersed. He termed this predator satiation, a phenomenon previously observed in periodical cicadas. Center & Johnson (1974) presented examples which presumably showed that bruchids were able to counter the defense of predator satiation with countermeasures of their own. Bruchids evolved the ability to feed in smaller seeds (i.e., became smaller in body size) and fed upon more than one seed during their ontogenetic development. Nelson & Johnson (1983a) found that in *Astragalus* there was stabilizing selection for an optimal seed size. Their data suggested that bruchids in species of *Astragalus* prefer large seeds over smaller seeds, but larger seeds had a better chance of germination. Their data also suggested that because of the opposing processes of predation rates and germination success, the intermediate seed-size classes were most likely to contribute most to the next generation.

OTHER DEFENSES

Janzen (1969) emphasized that plants had developed many defenses against seed predation but that "virtually all [defensive traits] seem effective against at least one species of bruchid but only

rarely against all bruchids." He suggested that legumes exhibit at least 31 "traits that may be functional in eliminating or lowering bruchid destruction of seeds." Center & Johnson (1974) discovered apparent countermechanisms to 11 of the traits evolved by the Leguminosae. Countermechanisms to defensive traits against bruchids, such as gum production by pods, dehiscence, indehiscence, flaking of pod surface, etc., were described.

SUCCESS OF BRUCHIDS IN HOSTS AND NONHOSTS

Much of the research on the abilities of bruchids to feed in the seeds of nonhost plants has tried to determine the ability of insects to penetrate thick, hard, or toxic seed coats, or to develop in presumably toxic seed contents. Janzen (1977) found that the bruchid *Callosobruchus maculatus* was able to develop in only one of 63 nonhost seeds it was exposed to. He showed that seed coats have a different effect on this bruchid than do the seed contents. He cautioned against treating seed coats the same as seed contents in studies of bruchid host preferences. Janzen et al. (1977) incorporated secondary compounds in the normal diets of the same species of bruchid. The results showed that alkaloids, amino acids, and a variety of other secondary compounds were toxic to *C. maculatus*. Janzen et al. concluded that many of the secondary compounds in seeds are likely to be toxic to at least some animals and are likely to be responsible to some extent for host specificity of seed-eating insects.

Johnson (1981d) exposed six species of the noneconomic bruchid genus *Stator* to a variety of nonhost seeds. He found that some species with a broad host range in nature could not feed in many nonhost seeds in the laboratory. Conversely, a species with a very narrow, specialized range of hosts in nature was found to be able to feed in a very broad range of nonhosts. The general rule, however, is that those with very narrow host ranges are not able to feed in nonhost seeds. Some of those with few natural hosts were very selective in the seeds they would oviposit on. Ovipositional behavior of the adult female apparently affects the ability of larvae to utilize seeds, since the larvae cannot move to an alternate host. Johnson also found that there is differential success of some species of *Stator* between different species of their natural hosts, and that seed coats are formidable barriers to larval entry of most species of *Stator*, even in their natural hosts.

IV. Coevolution

Much has been written about coevolution since Ehrlich & Raven coined the term in 1964. Because the term coevolution had been used in a variety of ways by different authors (e.g., that coevolution can occur between organisms and abiotic factors in their environment), Janzen (1980a) provided a definition for coevolution that is used by many today. No concise example of coevolution has been documented between insects and plants (Futuyma, 1983), although Janzen maintains that his work on ants and acacias is an example of coevolution (Janzen, 1967). Because of this, some authors are using the terminology plant-insect interactions rather than coevolution.

Janzen (1969) was the first to discuss coevolution of bruchids and legumes. Some of his data were discussed above, but the gist of his paper was that there has been a long period of coevolution between bruchids and legumes. He pointed out many traits possessed by the Leguminosae that can be interpreted as defenses against the seed-eating Bruchidae. His major findings were that toxic seeds and predator satiation were primary defenses against bruchids. Center & Johnson (1974) described examples of how bruchids probably had thwarted 11 of the defenses evolved by the Leguminosae, including toxins in seeds and predator satiation.

Johnson & Slobodchikoff (1979) presented data that they felt showed evidence of coevolution between *Cassia* (s.l.) and bruchids that feed in their seeds. Their evidence was that species of bruchids that feed in seeds of species of *Cassia* can be divided into specialist and generalist

species. Most (82.5%) of these bruchids are specialist species and feed on 1–3 species of host plants. The few generalist species feed on 5–10 species of host plants, all are homodynamic, most feed on many of the same host plant species, all have a broad geographic range, and all feed in a broader range of seed volumes than the specialist species. Johnson & Slobodchikoff hypothesized that seeds of species of *Cassia* that host 5–7 bruchid species have few toxins, but the species of *Cassia* that host 1–3 species of bruchids are very toxic.

V. Distribution of Hosts and Bruchids

Futuyma (1983) indicated that the number and composition of species of insects on a plant is due to a number of factors, but one of the most important is the geographical range of the plant. Evidence for this in the interactions between bruchids and legumes was presented by Johnson & Slobodchikoff (1979) and Johnson (1981b, 1983a). These authors and Janzen (1980b) presented data that showed that most bruchids have few hosts, and the seeds of most species of legumes that are hosts of bruchids are fed upon by only one species of bruchid. Johnson (1982) and Johnson & Janzen (1982) presented data to show that *Enterolobium cyclocarpum* (Mimosoideae) was fed upon by the bruchid *Stator generalis* only in Panama, although the tree occurs northward into central Mexico and southward into central South America. Johnson (1984) reported that the beetles feed on the seeds well into Venezuela, and I have since reared them from seeds in east central Venezuela, near the border with Guyana. These data show that ecological conditions north of Panama are not suitable to maintain populations of *S. generalis* but the conditions to the south are. The data also demonstrate that a plant with a broad range does not necessarily host numerous species of bruchids.

VI. Life History Strategies Of The Bruchidae

The life histories of a number of noneconomic bruchids have been described (e.g., Janzen, 1971; Forister & Johnson, 1970, 1971; Center & Johnson, 1973; Johnson & Kingsolver, 1971, 1975; Johnson & Janzen, 1982; Johnson, 1974, 1977a, b, 1981e). One of the more interesting aspects of these studies is the bruchid *Neltumius texanus*. This species feeds on several species in the rhamnaceous genus *Condalia*. Most interesting is that the other two species in the genus *Neltumius* are specific to different species in the legume genus *Prosopis*. Because the bruchid genus is thought to be monophyletic, it is probable that ancestors of the present-day species fed only on seeds of *Prosopis*, and a species subsequently evolved to feed in seeds of *Condalia*. This is thought to be the method by which the species of *Acanthoscelides* have come to feed in the seeds of non-legumes.

Janzen (1971) observed that the ability of bruchids to feed in the seeds of *Cassia grandis* was a complex interaction where at least one species of bruchid was dependent upon another species of bruchid and moth larvae to open entry holes into the seed pod and to clean the seeds before oviposition could occur.

Johnson (1981c) reported that several species of bruchids in the genus *Stator* feed on seeds only after they have fallen to the substrate. This information coupled with previously described or observed behavior patterns of many other species of bruchids enabled Johnson to describe three guilds of bruchid beetles. He found that the genera in guild 1 (oviposits on legume pods) are different from the genus (guild 2), the members of which oviposit on seeds while they are still inside a partially dehiscent pod while it is on the tree. Guild 3 is the group that oviposits only on seeds on the ground. The three patterns of pod behavior are indehiscent pods (fed upon by guilds 1 and 3), partially dehiscent pods (fed upon by guilds 1, 2, and 3), and dehiscent pods (fed upon by guild 3, sometimes by guild 2).

With this information it would appear that plants with partially dehiscent pods should lose a greater number of seeds to bruchids, and thus if bruchids have a significant evolutionary impact on

the methods of seed dispersal by plants, plants with this mode of behavior should be fewer in number than those with dehiscent or indehiscent pods. This was not the case with the species of legumes that Johnson studied. Indeed, he found that there were more species (21) with partially dehiscent pods than the other two categories combined (15). He attributed the patterns to be a result of combined selective pressure for seed dispersal and for protection from seed predators.

VII. Economic Importance Of The Bruchidae

Most bruchids of economic importance feed in stored seeds. The bean weevil *(Acanthoscelides obtectus)* is the most important of the bruchid beetles that feed in the seeds of legumes used for food by humans because it feeds in species of *Phaseolus*. Several other species are economically important in grain legumes, including those that feed in stored peanuts. The storing of different species of grain legumes in the same warehouse has led to some interesting host switches (and host records) by bruchids of economic importance.

Bruchids feed in a great variety and number of legumes of no contemporary economic importance. However, because of these feeding habits, they are of considerable importance to scientists who wish to propagate these seeds for their studies.

Recently the Food and Agricultural Organization of the United Nations embarked on a project to breed better varieties of species of *Acacia* and *Prosopis* for use as fuelwood in underdeveloped countries. Studies of the bruchids that feed in the seeds of these genera have been published by Southgate (1983a, b, c) and Johnson (1983b, c, d) because they feed in seeds used for propagation. Many species of bruchids may become economically important when legumes that are not now important in the economy become so in the future. Also, legumes that become weeds in the future may be controlled in part by bruchids.

VIII. Parasites

Bruchids are attacked by a variety of parasites, primarily those in the insect order Hymenoptera. These parasites have been studied infrequently but could be of major significance to studies of economic and noneconomic bruchids alike. Their importance to the possible control of economic bruchids is obvious.

IX. Adaptive Radiation of *Acanthoscelides*

Very little is known about the mechanisms responsible for host specificity in species of *Acanthoscelides*. Many of the factors mentioned above are most likely responsible for host preferences, but at this time very little is known about specific bruchids and their preferences for seeds of certain plants.

Table 2. Plants fed upon by species of *Acanthoscelides*.

Host plants	*Acanthoscelides* species
LEGUMINOSAE	
Acacia collinsii	*petalopygus*
A. cornigera	*oblongoguttatus*
	sousai
A. cornigera + *A. sphaerocephala*	*oblongoguttatus*
A. gentlei	*sousai*

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
A. sphaerocephala	*oblongoguttatus*
Aeschynomene americana	*megacornis*
	pertinax
	modestus
A. ciliata	*modestus*
A. rudis	*modestus*
A. scabra	*modestus*
A. sensitiva	*pallidipennis*
Amorpha californica	*submuticus*
	pallidipennis
A. canescens	*floridae*
A. fruticosa	*pallidipennis*
	submuticus
	submuticus
A. fruticosa angustifolia	*submuticus*
A. fruticosa occidentalis	*ambopygus*
Apuleia leiocarpa	*bilobatus*
	unguiculatus
	aureolus
Astragalus allochrous	*mixtus*
	pullus
	aureolus
A. antiselli	*pullus*
	aureolus
A. asymmetricus	*pullus*
	aureolus
A. bisulcatus	*fraterculus*
	pullus
A. bolanderi	*aureolus*
A. calycosus	*perforatus*
A. canadensis	*fraterculus*
A. caryocarpus	*fraterculus*
A. cibarius	*fraterculus*
A. crassicarpus	*aureolus*
A. crotalariae	*pullus*
	aureolus
A. douglasii	*pullus*
	aureolus
A. drummondii	*fraterculus*
	aureolus
A. fucatus	*aureolus*
A. grayii	*aureolus*
A. humistratus	*lobatus*
	fraterculus
A. hyalinus	*aureolus*
A. lentiginosus	*lobatus*
	mixtus
	pullus
	fraterculus
	aureolus
A. missouriensis	*fraterculus*
A. mollissimus	*lobatus*

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
A. mollissimus var. *bigelovii*	*lobatus*
A. mollissimus var. *marcidus*	*lobatus*
A. oxyphysus	*aureolus*
	pullus
A. pattersoni	*aureolus*
	fraterculus
A. pectinatus	*fraterculus*
A. praelongus	*aureolus*
	mixtus
	pullus
A. praelongus var. *lonchopus*	*mixtus*
A. pycnostachyus	*margaretae*
A. racemosus	*aureolus*
	fraterculus
A. sabulonum	*mixtus*
A. thurberi	*mixtus*
	pullus
A. trichopodus	*pullus*
A. troglodytus	*lobatus*
A. utahensis	*fraterculus*
A. wootoni	*aureolus*
	mixtus
	pullus
Cajanus bicolor	*zeteki*
C. cajan	*zeteki*
Calopogonium caeruleum	*hectori*
	taboga
C. mucunoides	*griseolus*
	puellus
	taboga
Chaetocalyx latisiliqua	*mundulus*
Cicer arietinum	*obtectus*
Dalea aurea	*seminulum*
D. bicolor var. *naviculifolia*	*pusillimus*
D. carthagenensis var. *barbata*	*pusillimus*
D. cliffortiana	*pusillimus*
D. enneandra	*seminulum*
D. fremontii	*daleae*
D. frutescens	*seminulum*
D. leporina	*pertinax*
	pusillimus
D. leucostachya var. *eysenhardtioides*	*pusillimus*
D. scandens var. *occidentalis*	*pusillimus*
D. scandens var. *paucifolia*	*pusillimus*
D. schottii	*daleae*
D. spinosa	*daleae*
Dalea aff. *submontana*	*pertinax*
	pusillimus
D. tomentosa var. *tomentosa*	*pusillimus*
D. zimapanica	*pusillimus*
Desmanthus acuminata	*compressicornis*

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
	compressicornis
D. cooleyi	*compressicornis*
D. covillei	*desmanthi*
	derifieldi
	compressicornis
D. depressus	*compressicornis*
D. illinoensis	*bisignatus*
	compressicornis
D. leptolobus	*bisignatus*
	compressicornis
D. obtusus	*pectoralis*
	compressicornis
D. subulatus	*derifieldi*
	compressicornis
D. velutinus	*bisignatus*
	pectoralis
	compressicornis
D. virgatus	*desmanthi*
	pectoralis
	puelliopsis
Desmodium adscendens	*desmoditus*
D. amplifolium	*desmoditus*
D. angustifolium	*puelliopsis*
D. barbatum	*biustulus*
D. batocaulon	*biustulus*
D. cinerascens	*desmoditus*
D. cinereum	*howdenorum*
	mazatlan
	megacornis
	cuernavaca
D. distortum	*desmoditus*
	mazatlan
	mazatlan
D. glabrum	*biustulus*
D. grahamii	*stylifer*
	biustulus
D. hartwegianum	*puelliopsis*
D. incanum	*schubertae*
	puelliopsis
D. intortum var. *intortum*	*biustulus*
D. neomexicanum	*desmoditus*
D. nicaraguense	*biustulus*
D. nitidum	*cuernavaca*
D. orbiculare	*desmoditus*
D. orbicularis	*desmoditus*
D. orbicularis var. *salvini*	*desmoditus*
D. orbicularis var. *rubricaule*	*desmoditus*
D. rensoni	*mazatlan*
D. scorpiurus	*desmoditus*
D. skinneri	*cuernavaca*
D. tortuosum	*desmodicola*

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
	desmoditus
	mazatlan
	megacornis
	pertinax
Dolichos lablab	*argillaceus*
Eriosema grandiflorum	*eriosemicola*
E. violaceum	*flavescens*
Errazurizia rotundata	*pallidipennis*
Flemingia strobilifera	*zeteki*
Galactia striata	*flavescens*
G. wrightii	*rufovittatus*
Glycyrrhiza lepidota	*aureolus*
	fraterculus
Hoffmanseggia densiflora	*compressicornis*
H. drepanocarpa	*compressicornis*
H. tenella	*compressicornis*
Indigofera costaricensis	*kingsolveri*
I. densiflora	*kingsolveri*
	ruficoxis
I. lindheimeriana	*kingsolveri*
I. panamensis	*kingsolveri*
I. platycarpa	*baboquivari*
	kingsolveri
I. sphaerocarpa	*baboquivari*
	kingsolveri
I. suffruticosa	*indigoferestes*
	kingsolveri
Lathyrus sativus	*obtectus*
Lens esculenta	*obtectus*
Lespedeza capitata	*longistilus*
L. hirta	*longistilus*
L. texana	*longistilus*
L. virginica	*longistilus*
Leucaena collinsii	*macrophthalmus*
	mankinsi
Leucaena aff. *collinsii*	*macrophthalmus*
L. diversifolia	*boneti*
	leucaenicola
L. esculenta	*boneti*
	leucaenicola
	macrophthalmus
	mankinsi
L. glauca	*macrophthalmus*
	mankinsi
L. guatemalensis	*macrophthalmus*
L. lanceolata	*leucaenicola*
	macrophthalmus
	mankinsi
L. leucocephala	*macrophthalmus*
	mankinsi
L. macrophylla	*macrophthalmus*
	mankinsi

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
	macrophthalmus
L. pulverulenta	*macrophthalmus*
L. retusa	*macrophthalmus*
L. shannoni	*mankinsi*
	macrophthalmus
L. salvadorensis	*fraterculus*
Lotus crassifolius	*fraterculus*
L. mearnsii	*aureolus*
L. purshianus	*fraterculus*
L. rigidus	*aureolus*
L. scoparius	*hectori*
Macroptilium atropurpureum	*chiricahuae*
Mimosa aculeaticarpa	*chiricahuae*
M. albida	*cordifer*
	difficilis
	mimosicola
	mexicanus
M. near *albida*	*schrankiae*
	difficilis
M. albida var. *strigosa*	*chiricahuae*
M. arida	*schrankiae*
M. bahamensis	*chiricahuae*
M. biuncifera	*mexicanus*
	speciosus
	chiricahuae
M. borealis	*chiricahuae*
M. brandegei	*mexicanus*
	piceoapicalis
M. casta	*chiricahuae*
M. depauperata	*nigronotaticeps*
M. detinens	*devriesi*
M. dormiens	*puniceus*
	chiricahuae
M. dysocarpa	*mexicanus*
M. egregia	*chiricahuae*
M. emoryana	*mexicanus*
M. eurycarpa	*schrankiae*
	mexicanus
M. eurycarpoides	*schrankiae*
	nigronotaticeps
M. farinosa	*mexicanus*
M. galeottii	*chiricahuae*
M. grahamii	*cordifer*
M. guanacastensis	*difficilis*
	schrankiae
M. hemiendyta	*piceoapicalis*
M. invisa	*chiricahuae*
M. lacerata	*mexicanus*
	cordifer
M. langlassei	*difficilis*
	mimosicola

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
M. laxiflora	*chiricahuae*
	mexicanus
M. malacophylla	*speciosus*
M. monancistra	*chiricahuae*
M. palmeri	*chiricahuae*
	mexicanus
M. panamensis	*lapsanae*
M. pigra	*difficilis*
	pigricola
	puniceus
	quadridentatus
	zebratus
M. pigra var. *berlandieri*	*quadridentatus*
M. platycarpa	*mexicanus*
M. polyanthoides	*mexicanus*
	schrankiae
M. pudica	*difficilis*
	lapsanae
	mimosicola
	piceoapicalis
M. pusilla	*difficilis*
M. quadrivalvis	*piceoapicalis*
	schrankiae
M. setigera	*mexicanus*
	schrankiae
M. somnians	*piceoapicalis*
	tridenticulatus
M. strigillosa	*quadridentatus*
	tridenticulatus
M. velloziana	*cordifer*
	difficilis
Nissolia fruticosa	*mundulus*
N. schottii	*mundulus*
Oxytropis lambertii	*aureolus*
O. sericea	*aureolus*
Pachyrhizus erosus	*sanfordi*
	taboga
Parkia cachimboensis	*aurulentus*
P. decussata	*aurulentus*
	helenae
	hopkinsi
	lambda
	phalaropus
P. discolor	*aurulentus*
	bisagittus
	hopkinsi
	phalaropus
P. igneiflora	*aurulentus*
	bisagittus
	hopkinsi
P. multijuga	*hopkinsi*
	lambda

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
P. nitida	*aurulentus*
	hopkinsi
	phalaropus
P. panurensis	*aurulentus*
	bisagittus
	hopkinsi
	phalaropus
P. pendula	*imitator*
P. platycephala	*imitator*
P. ulei	*aurulentus*
	hopkinsi
	phalaropus
P. velutina	*hopkinsi*
Parryella filifolia	*pallidipennis*
Petalostemum candidum	*seminulum*
P. feayi	*seminulum*
P. ornatus	*oregonensis*
P. stanfieldii	*seminulum*
Petalostemum sp.	*pedicularius*
Phaseolus aconitifolius	*obtectus*
P. acutifolius	*obtectus*
P. acutifolius var. *latifolius*	*obtectus*
P. aureus	*obtectus*
P. calcaratus	*obtectus*
P. caracalla	*obtectus*
	caracallae
P. coccineus	*obtectus*
P. glabellus	*obvelatus*
P. lunatus	*argillaceus*
	obtectus
P. vulgaris	*clandestinus*
	obtectus
	obvelatus
	comptus
Phaseolus aff. *peduncularis*	*clitellarius*
Piptadenia flava	*obtectus*
Pisum sativum	*longescutus*
Prosopis strombulifera	*distinguendus*
Rhynchosia americana	*isla*
R. calycosa	*zeteki*
	distinguendus
R. difformis	*sanfordi*
Rhynchosia aff. *yucatanensis*	*amabilis*
R. discolor	*distinguendus*
R. erecta	*distinguendus*
R galactioides	*distinguendus*
R. intermedia	*distinguendus*
R. latifolia	*campeche*
R. longeracemosa	*flavescens*
	palmasola
	zeteki

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
R. minima	*flavescens*
	hectori
	zeteki
R. phaseoloides	*flavescens*
	rhynchosiestes
R. pyramidalis	*rhynchosiestes*
	zeteki
R. reticulata	*campeche*
	surrufus
	zeteki
R. texana	*fumatus*
R. tomentosa	*distinguendus*
Schrankia diffusa	*schrankiae*
S. distachya	*schrankiae*
S. microphylla	*schrankiae*
S. paucijuga	*schrankiae*
S. roemeriana	*schrankiae*
S. uncinata	*schrankiae*
Senna armata	*obrienorum*
S. atomaria	*obrienorum*
S. biflora	*obrienorum*
S. pallida	*obrienorum*
S. pallida var. *gaumeri*	*obrienorum*
S. polyantha	*obrienorum*
S. racemosa	*obrienorum*
S. skinneri	*obrienorum*
S. villosa	*obrienorum*
S. wislizenii	*obrienorum*
Sesbania aegyptiaca	*obtectus*
S. emerus	*griseolus*
S. exaltata	*griseolus*
S. macrocarpa	*griseolus*
Stylosanthes sp. prob. *humilis*	*pertinax*
Tephrosia ambigua	*obsoletus*
T. cana	*rufovittatus*
T. cinerea	*rufovittatus*
T. multifolia	*rufovittatus*
T. rhodantha	*rufovittatus*
T. spicata	*obsoletus*
T. tenella	*rufovittatus*
T. thurberi	*rufovittatus*
T. virginiana	*obsoletus*
Teramnus labialis	*zonensis*
T. uncinatus	*argutus*
T. volubilis	*argutus*
	zonensis
Trifolium obtusiflorum	*pauperculus*
T. pratense	*pusillimus*
	pyrrhomelas
Vicia faba	*obtectus*
Vigna peduncularis	*suaveolus*

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
V. sesquipedalis	*obtectus*
V. unguiculata (=V. sinensis)	*obtectus*
V. vexillata	*suaveolus*
Vigna sp.	*caracallae*
ANACARDIACEAE	
Lithraea brasiliensis	*atronotatus*
CISTACEAE	
Helianthemum canadense	*calvus*
H. corymbosum	*helianthemum*
Lechea tenuifolia	*atomus*
LYTHRACEAE	
Lythrum alatum	*tenuis*
L. lineare	*tenuis*
MALVACEAE	
Abutilon amplexifolium	*tepic*
A. andrieuxii	*sleeperi*
A. berlandieri	*subaequalis*
A. californicum	*aequalis*
	subaequalis
	aequalis
A. incanum	*subaequalis*
	subaequalis
A. lignosum	*sleeperi*
A. palmeri	*subaequalis*
A. pringlei	*aequalis*
A. trisulcatum	*subaequalis*
	merida
A. umbellatum	*malvitus*
Abutilon sp.	*anoditus*
Anoda cristata	*guerrero*
Herissantia crispa	*herissantitus*
	johni
	elkinsae
Hibiscus sp.	*hibiscicola*
	fryxelli
Kosteletzkya depressa	*orlandi*
	fryxelli
K. hispidula	*blanchardi*
K. tubiflora	*fryxelli*
Malachra capitata	*orlandi*
	apicalis
Malachra spp.	*fryxelli*
	orlandi
	malvitus
Malva sp.	*brevipes*
Malvastrum americanum	*guerrero*
	malvastrumicis
	brevipes
M. bicuspidatum	*herissantitus*
	brevipes
M. corchorifolium	*pavoniestes*
Pavonia candida	

TABLE 2. Continued.

Host plants	*Acanthoscelides* species
Pseudabutilon lozani	*aequalis*
Sida acuta	*brevipes*
S. aggregata	*brevipes*
S. pyramidata	*pyramididos*
S. rhombifolia	*brevipes*
S. spinosa	*brevipes*
Wissadula amplissima	*aequalis*
W. holosericea	*aequalis*
ONAGRACEAE	
Ludwigia alternifolia	*alboscutellatus*
L. palustris	*alboscutellatus*
RHAMNACEAE	
Ziziphus obtusifolia	*prosopoides*
STERCULIACEAE	
Guazuma ulmifolia	*guazumae*
TILIACEAE	
Heliocarpus attenuatus	*sanblas*
H. palmeri	*cornis*
	johnsoni
H. popayanensis	*johnsoni*
Triumfetta bogotensis	*manducus*
T. calderoni	*johnsoni*
	sanblas
	triumfettae
T. lappula	*bellus*
	johnsoni
	sanblas
	triumfettae
T. paniculata	*bellus*
T. semitriloba	*sanblas*
T. speciosa	*sanblas*

SPECIES GROUPS OF *ACANTHOSCELIDES*

In this section the adaptive radiation of species of *Acanthoscelides* will be discussed. Most host records and species groups of *Acanthoscelides* were taken from Johnson (1983a) and Kingsolver (1982, 1984) and are listed in Tables 1 and 2.

Acanthoscelides is the largest genus of Bruchidae in the New World. At this time it is a genus composed of many disparate but related entities. No doubt its large size is due to an adaptive radiation of one or more elements into the seeds of the Leguminosae and other host families. The general pattern of radiation is that most species feed in seeds of the Papilionoideae, Mimosoideae, or Malvaceae, with an occasional species feeding in the seeds of the Caesalpinioideae or another family. For example, 15 of the 16 species in group 12 (Johnson, 1983a) feed only in seeds of the Malvaceae and the other species feeds in the closely related family Sterculiaceae; Groups 8, 10, and 13 only in the Mimosoideae, and Groups 1, 2, and 7 only in the Papilionoideae.

Groups 1 *(argillaceus, eriosemicola, obtectus, obvelatus)* and 2 *(pusillimus)* (Table 1) feed only in the seeds of papilionoid legumes. (The number of a species group generally indicates the relationship of species groups, i.e., close numbers indicate a close morphological relationship.) *A. obtectus* is the common bean weevil and has become cosmopolitan through commerce. Because of

its distribution it also has become exposed to many other stored legume seeds and feeds in them as well. Its two close relatives, *A. argillaceus* and *A. obvelatus*, feed primarily in seeds of *Phaseolus*. *A. eriosemicola* is known only to feed in seeds of *Eriosema*.

Species Group 2 is composed only of the unique *A. pusillimus*, which feeds primarily in *Dalea* but has been reported from the seeds of *Trifolium*.

The seeds of *Mimosa* are the only food for members of Group 3 (*A. quadridentatus* and its relatives) except that *A. pectoralis* feeds in the seeds of *Desmanthus* and *A. triumfettae* feeds in the seeds of *Triumfetta* (Tiliaceae). An ancestor of this group switched to the Tiliaceae. Seeds of *Mimosa* are fed upon by 16 species of *Acanthoscelides* and several other species of the Bruchidae. *Mimosa* is a large genus that has been exploited very successfully by bruchids. By contrast, the much larger genus *Acacia* has numerous bruchids in its seeds, but only a very small percentage of seeds are fed upon.

The 26 members of Group 4 (*A. puellus* and its relatives) feed only in papilionoid legumes except for *A. prosopoides* and a relative that feeds in seeds of the Rhamnaceae. *A. prosopoides* has moved into the seeds of the Rhamnaceae, as have other species of *Acanthoscelides* into other families. Six members of the Flavescens Group (5) feed in seeds of papilionoid legumes, but *A. compressicornis* feeds in seeds of both the Mimosoideae and the Caesalpinioideae (Tables 1, 2). It is the only *Acanthoscelides* known to feed in more than one subfamily of legumes. No species of *Acantho-scelides* is known to feed in more than one family of plants. *A. alboscutellatus* feeds in the seeds of two species of Onagraceae, another example of one species evolving the ability to feed in the seeds of a non-legume.

Five of the seven members of Group 6 (*A. blanchardi* and its relatives) have known hosts and all five feed in the Malvaceae. These species are an excellent example of adaptive radiation into a non-legume host family because all five are closely related in their morphology to other typical *Acanthoscelides*. They are unlike the members of Group 12, which feed in another group of the Malvaceae but are marginal members of *Acanthoscelides*.

All species in Group 7 (the Pertinax Group) feed in papilionoid legumes. Most are specific to *Desmodium* but some feed in *Teramnus*, *Petalostemon*, *Dalea*, and *Aeschynomene*. This is another example of adaptive radiation into a specific group of Leguminosae. Based on their morphology, all are closely related and apparently evolved from a common ancestor that exploited the seeds of these legumes.

Only two of the six members of Group 8 (the Albopygus Group) have known hosts. Both *A. petalopygus* and *A. sousai* feed in seeds of *Acacia*.

Most of the members of Group 9 (*A. mexicanus* and its relatives) feed in seeds of the Mimosoideae. Two members, however, feed in the Caesalpinioideae. *A. bilobatus* exploits the seeds of *Apuleia*, and *A. obrienorum* is the only species of *Acanthoscelides* known to feed in seeds of *Cassia* (s. l.). The species that feed in seeds of mimosoid legumes feed primarily in *Mimosa*, *Parkia*, and *Leucaena* (Tables 1, 2). *Desmanthus* also has been exploited by members of this group. Group 10 is composed of two highly specialized species that feed only in seeds of *Mimosa* and *Schrankia*. *A. chiricahuae* feeds in *Mimosa* while *A. schrankiae* feeds in *Mimosa* and is the only *Acanthoscelides* to feed in *Schrankia*. Recent collections in northern South America indicate that either these species or other closely related species infest the seeds of *Mimosa* and *Schrankia* there. Thus, this is a group that is specialized to use the seeds of two closely related genera.

Group 11 is composed of species that are related morphologically but feed in seeds of both the Leguminosae and the Tiliaceae. Like other species of *Acanthoscelides*, the ancestor of these species presumably fed in the Leguminosae but at least five of seven then radiated into the seeds of the Tiliaceae. The only other species known to use seeds of the Tiliaceae is *A. triumfettae*, a member of Group 3. *A. megacornis* feeds in the seeds of *Aeschynomene* and *Desmodium*, while *A. modestus* only feeds in *Aeschynomene*.

The 16 members of Group 12 are related morphologically and 15 feed in the seeds of the Malvaceae. The exception, *A. guazumae*, feeds in the seeds of Sterculiaceae (Table 1), a family

closely related to the Malvaceae. Most of these species are so similar in their external morphology that they can only be separated with certainty by examining the male genitalia. This group is an excellent example of adaptive radiation of bruchids into a unique group of hosts. There are no other species in *Acanthoscelides* that appear to be closely related to species in this group. They are not closely related to other species of *Acanthoscelides* that feed in the seeds of Malvaceae (Group 6). In many of their features they are related to the single species in the genus *Abutiloneus*.

A. oblongoguttatus is the only member of Group 13. It has many characteristics that link it to other *Acanthoscelides*, but it is also related to other genera of Bruchidae. It feeds only in *Acacia* but is not close to other *Acanthoscelides* that feed in seeds of *Acacia*.

A. mundulus feeds in seeds of papilionoid legumes but is an aberrant *Acanthoscelides*. Thus its host preferences offer few clues to the adaptive radiation of the genus.

The general pattern of host selection (adaptive radiation) in species of *Acanthoscelides* is that most species groups feed in a genus or a subfamily of legumes with an occasional species or group of species adapting to and exploiting another subfamily of legumes or family of plants.

PERCENT OF SPECIES OF PLANTS ATTACKED

Bruchids usually tend to be specialized to one or a few hosts in a localized area, and wide-ranging species of plants are attacked by different bruchids in different parts of their range. Examples of this are explored below.

Table 3. Percentage of species of plants fed upon by species of *Acanthoscelides*, percentage of species of *Acanthoscelides* feeding in host plants, and ratios of numbers of plant species fed upon to number of species in the plant taxon.

Host	Number of plant species fed upon by *Acanthoscelides*	Number of species of *Acanthoscelides*
Caesalpinioideae		
Apuleia	1	3
Hoffmanseggia	3	1
Senna	9	1
Totals	13	5
% of subfamily fed upon/legume = 4.9		
% of subfamily fed upon/total plants = 4.1		
% *Acathoscelides* spp. in subfamily of legumes = 3.7		
% *Acanthoscelides* spp. in subfamily compared to total number of plants = 2.8.		
Ratio of number of plant species that are hosts to bruchids to total species of plants in subfamily. 2,500/13 = 1:192		
Mimosoideae		
Acacia	4	3
Desmanthus	10	5
Leucaena	12	4
Mimosa	39	16
Parkia	10	7
Piptadenia	1	1
Prosopis	1	1
Schrankia	6	1
Totals	83	38
% of subfamily fed upon/legume = 31.3		
% of subfamily fed upon/total plants = 26.3		
% *Acanthoscelides* spp. in subfamily of legumes = 27.7		
% *Acanthoscelides* spp. in subfamily compared to total number of plants = 21.2		
Ratio: 2,500/83 = 1:30		

Table 3. Continued.

Host	Number of plant species fed upon by *Acanthoscelides*	Number of species of *Acanthoscelides*
Papilionoideae		
Aeschynomene	5	3
Amorpha	3	3
Astragalus	32	7
Cajanus	2	1
Calopogonium	2	4
Chaetocalyx	1	1
Cicer	1	1
Dalea	15	4
Desmodium	22	10
Dolichos	1	1
Eriosema	2	2
Errazurizia	1	1
Flemingia	1	1
Galactia	2	2
Glycyrrhiza	1	2
Indigofera	7	4
Lathyrus	1	1
Lens	1	1
Lespedeza	4	1
Lotus	5	2
Macroptilium	1	1
Nissolia	2	1
Oxytropis	2	1
Pachyrhizus	1	2
Parryella	1	1
Petalostemum	4	3
Phaseolus	9	6
Pisum	1	1
Rhynchosia	16	12
Sesbania	4	2
Stylosanthes	1	1
Tephrosia	9	2
Teramnus	3	2
Trifolium	2	3
Vicia	1	1
Vigna	5	3
Totals	169	94
Legume Total	265	137
Ratio (subfamily): 12,000/169 = 1:71		
Ratio (Leguminosae): 17,000/265 = 1:64		
% of subfamily fed upon/legume = 63.8		
% of subfamily fed upon/total plants = 53.5		
% *Acanthoscelides* spp. in subfamily of legumes = 68.6		
% *Acanthoscelides* spp. in subfamily compared to total number of plants = 52.2		
% of Total Records (legumes) = 76.1		
ANACARDIACEAE		1
Lithraea	1	
Ratio: 600/1 = 1:600		
% of family fed upon/total plants = 0.3		
% *Acanthoscelides* spp. in family compared to total number of plants = 0.5		

Table 3. Continued.

Host	Number of plant species fed upon by *Acanthoscelides*	Number of species of *Acanthoscelides*
CISTACEAE		
Helianthemem	2	2
Lechea	1	1
Totals	3	3
Ratio: 160/3 = 1:53		
% of family fed upon/total plants = 0.9		
% *Acanthoscelides* in family compared to total number of plants = 1.7		
LYTHRACEAE		
Lythrum	2	1
Ratio: 550/2 = 1:275		
% of family fed upon/total plants = 0.6		
% *Acanthoscelides* in family compared to total number of plants = 0.5		
MALVACEAE		
Abutilon	11	5
Anoda	1	1
Herissantia	1	3
Hibiscus	1	2
Kosteletzkya	3	3
Malachra	2	3
Malva	1	1
Malvastrum	3	4
Pavonia	1	1
Pseudabutilon	1	1
Sida	5	2
Wissadula	2	1
Totals	32	27
Ratio: 1,000/20 = 1:50		
% of family fed upon/total plants = 10.1		
% *Acanthoscelides* spp. in family compared to total number of plants = 15		
ONAGRACEAE		
Ludwigia	2	1
Ratio: 600/1 = 1:600		
% of family fed upon/total plants = 0.6		
% *Acanthoscelides* spp. in family compared to total number of plants = 0.5		
RHAMNACEAE		
Ziziphus	1	1
Ratio: 600/1 = 1:600		
% of family fed upon/total plants = 0.3		
% *Acanthoscelides* spp. in family compared to total number of plants = 0.5		
STERCULIACEAE		
Guazuma	1	1
Ratio: 700/1 = 1:700		
% of family fed upon/total plants = 0.3		
% *Acanthoscelides* spp. in family compared to total number of plants = 0.5		
TILIACEAE		
Heliocarpus	3	3
Triumfetta	6	5
Ratio: 450/6 = 1:75		
Totals	9	8
% of family fed upon/total plants = 2.8		
% *Acanthoscelides* spp. in family compared to total number of plants = 4.4		
Non-legume Total =	51	43
Grand Total =	316	180

TABLE 4. Absolute numbers, ratios, and percentages of species of *Acanthoscelides* that feed in seeds of various host families and subfamiles.

Family	Number of species	Ratios	% feeding in taxon
Leguminosae			
Papilionoideae	62	1:193	45.3
Mimosoideae	36	1:69	26.3
Caesalpinioideae	5	1:500	3.7
Subtotal (legumes)	103	1:165	75.2
Anacardiaceae	1	1:600	0.7
Cistaceae	3	1:53	2.2
Lythraceae	1	1:550	0.7
Malvaceae	20	1:50	14.6
Onagraceae	1	1:600	0.7
Rhamnaceae	1	1:600	0.7
Sterculiaceae	1	1:700	0.7
Tiliaceae	6	1:75	4.4
Subtotal (non-legumes)	34	1:137	24.8
TOTAL	137	1:158	

Over their entire range, *Mimosa* and *Astragalus* have been exploited most by species of *Acanthoscelides*, with 39 and 32 species respectively being fed upon (Table 3). The other plant genera that have many of their species fed upon are *Desmodium* with 22 species fed upon; *Dalea* with 15 species; *Rhynchosia* with 16 species; *Leucaena* with 12 species; and *Desmanthus* and *Parkia* with 10 species each. The general pattern is that only a few species of *Acanthoscelides* feed in a rather large number of plant species (e.g., *Astragalus* exhibits this phenomenon and probably more species would also if an extensive survey were made). The reverse is true, however, with *Parkia*. Hopkins (1983) discussed the unusual circumstance that in the Amazon Basin many bruchids feed in a single host and many hosts are used by the same species of bruchids.

Most of the host records for *Acanthoscelides* are in the Papilionoideae (76.1%; Table 3). The records in the Mimosoideae account for 21.2% and those in the Malvaceae 15.1%. Surprisingly, there is a greater percent of records in the Tiliaceae (4.4%) than in the Caesalpinioideae (2.8%). The Caesalpinioideae are attacked less frequently by the Bruchidae than are the other subfamilies of the Leguminosae (Johnson, 1981a) so the fact that they are not fed upon by *Acanthoscelides* is not so surprising. What is unusual is that the families Tiliaceae and especially Malvaceae account for such a large percentage of hosts.

When absolute numbers of species of *Acanthoscelides* are used to calculate ratios of numbers of bruchid species to the numbers of species of plants worldwide in each subfamily or family, the results are quite different (Table 4). Papilionoideae (ca. 12,000 spp.) 1 *Acanthoscelides*:193 spp. papilionoid legume species, and 45.3%; Mimosoideae (ca. 2,500 spp.) 1:69, and 26.3%; and Caesalpinioideae (ca. 2,500 spp.) 1:500, and 3.7%. Using the same technique the ratios in Cistaceae (ca. 160 spp.) are 1:53, 2.2%; Malvaceae (ca. 1,000 spp.) 1:50, and 14.6%; and Tiliaceae (ca. 450 spp.) 1:75, 4.4%. Thus, two of these three families support more species of *Acanthoscelides* per number of species in each taxon than do two of the legume subfamilies. Only the subfamily Mimosoideae supports more than the Tiliaceae. Species of *Acanthoscelides* (and other bruchids) have sampled the seeds of many species of plants, both legumes and non-legumes, and have been able to feed in some more readily than others. Reasons for this were discussed above. The Mimosoideae, Malvaceae, and Tiliaceae are less able to defend their seeds from bruchids than are other taxa; thus these small families react to bruchid predation as do some of the large genera (e.g., *Mimosa, Acacia*) of the huge family Leguminosae that are especially susceptible to bruchid attack.

When the number of plant species fed upon by species of *Acanthoscelides* is divided into the total number of plant species in a subfamily or family, a ratio of number of species per taxon is obtained (Table 3). The results of this are also interesting. The Mimosoideae have one in 30 species fed upon by *Acanthoscelides,* but the ratio in the Malvaceae is one in 31. Thus for the total number of species per taxon, the Malvaceae are fed upon almost as frequently as are the most heavily infested subfamily of legumes. The ratios for the Papilionoideae are 1:71, the Caesalpinioideae 1:192, and for the Leguminosae 1:64. Other non-legumes that have interesting ratios are the Cistaceae with a ratio of 1:53 and the Tiliaceae 1:50. When additional studies are done on all the hosts of all the Bruchidae it may well be found that a family other than the Leguminosae is more susceptible to feeding per species by bruchids. The legume subfamilies are fed upon by many other genera of Bruchidae, and in overall numbers, they are more prevalent in the Leguminosae than in other families. Thus the legumes are the "favorite" hosts of bruchids.

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References

Center, T. D. & C. D. Johnson. 1973. Comparative life histories of *Sennius* (Coleoptera: Bruchidae). Environ. Entomol. 2: 669–672.

_____ & _____. 1974. Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. Ecology 55: 1096–1103.

Ehrlich, P. R. & P. H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586–608.

Forister, G. W. & C. D. Johnson. 1970. Bionomics of *Merobruchus julianus* (Coleoptera: Bruchidae). Coleop. Bull. 24: 84–87.

_____ & _____. 1971. Behavior and ecology of *Acanthoscelides prosopoides* (Coleoptera: Bruchidae). Pan-Pac. Entomol. 47: 224–234.

Futuyma, D. J. 1983. Evolutionary Interactions among Herbivorous Insects and Plants. Pp. 207–231 *in* D. J. Futuyma & M. Slatkin (editors), Coevolution. Sinauer Associates Inc., Sunderland, Massachusetts.

Hopkins, M. J. G. 1983. Unusual diversities of seed beetles (Coleoptera: Bruchidae) on *Parkia* (Leguminosae: Mimosoideae) in Brazil. Biol. Linn. Soc. 19: 329–338.

Janzen, D. H. 1967. Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. Univ. Kansas Sci. Bull. 47(6): 315–558.

_____. 1969. Seed-eaters versus seed size, number, toxicity, and dispersal. Evolution 23: 1–27.

_____. 1971. Escape of *Cassia grandis* L. beans from predators in time and space. Ecology 52: 964–979.

_____. 1977. How southern cowpea weevil larvae (Bruchidae: *Callosobruchus maculatus*) die on nonhost seeds. Ecology 58: 921–927.

_____. 1980a. When is it coevolution? Evolution 34: 611–612.

_____. 1980b. Specificity of seed-attacking beetles in a Costa Rican deciduous forest. J. Ecol. 68: 929–952.

_____, H. B. Juster & E. A. Bell. 1977. Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle *Callosobruchus maculatus.* Phytochemistry 16: 223–227.

Johnson, C. D. 1970. Biosystematics of the Arizona, California, and Oregon species of the seed beetle genus *Acanthoscelides* Schilsky (Coleoptera: Bruchidae). Univ. Calif. Publ. Entomol. 59: 1–116.

_____. 1974. Ecology of two *Acanthoscelides* from *Indigofera,* with a description of a new species (Coleoptera: Bruchidae). J. Kansas Entomol. Soc. 47: 268–278.

_____. 1977a. Ecology and behavior of *Acanthoscelides mundulus* in seeds of *Nissolia schottii* (Coleoptera: Bruchidae; Leguminosae). Pan-Pac. Entomol. 53: 161–167.

_____. 1977b. Life history of *Ctenocolum janzeni* (Coleoptera: Bruchidae) in seeds of *Piscidia mollis* (Leguminosae). Coleop. Bull. 31: 313–318.

_____. 1981a. Seed Beetle Host Specificity and the Systematics of the Leguminosae. Pp. 995–1027 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

_____. 1981b. Relations of *Acanthoscelides* with their Plant Hosts. Pp. 73–81 *in* V. Labeyrie (editor), The Ecology of Bruchids Attacking Legumes (Pulses). Series Entomologica, Volume 19, The Hague.
_____. 1981c. Interactions between bruchid (Coleoptera) feeding guilds and behavioral patterns of pods of the Leguminosae. Environ. Entomol. 10: 249–253.
_____. 1981d. Host preferences of *Stator* (Coleoptera: Bruchidae) in non-host seeds. Environ. Entomol. 10: 857–863.
_____. 1981e. The bionomics of *Stator pygidialis* (Coleoptera: Bruchidae). Coleop. Bull. 35: 241–242.
_____. 1982. Survival of *Stator generalis* (Coleoptera: Bruchidae) in host seeds from outside its geographical range. J. Kansas Entomol. Soc. 55: 718–724.
_____. 1983a. Ecosystematics of *Acanthoscelides* of southern Mexico and Central America. Misc. Publ. Entomol. Soc. Amer. 56: 1–370.
_____. 1983b. Handbook on Seed Insects of *Prosopis* Species. Ecology, Control, and Identification of Seed-infesting Insects of New World *Prosopis* (Leguminosae). Food and Agriculture Organization of the United Nations.
_____. 1983c. Guide des Insectes Parasites des Semences de *Prosopis*. Ecologie, Moyens de Lutte, Identification. Organisation des Nations Unies pour L'Alimentation et L'Agriculture.
_____. 1983d. Manual sobre Insectos que Infestan la Semilla de *Prosopis* (Leguminosae). Ecologia, Control, e Identificacion de insectos del Nuevo Mundo que infestan la semilla de *Prosopis* (Leguminosae). Organizacion de las Naciones Unidas para la Agricultura y la Alimentacion.
_____. 1984. New host records and notes on the biology of *Stator* (Coleoptera: Bruchidae). Coleop. Bull. 38: 85–90.
_____ & D. H. Janzen. 1982. Why are the seeds of the Central American guanacaste tree *(Enterolobium cyclocarpum)* not attacked by bruchids except in Panama? Environ. Entomol. 11: 373–377.
_____ & J. M. Kingsolver. 1971. Descriptions, life histories, and ecology of two species of Bruchidae infesting guacima in Mexico (Coleoptera). J. Kansas Entomol. Soc. 44: 141–152.
_____ & _____. 1973. A revision of the genus *Sennius* of North and Central America (Coleoptera: Bruchidae). Techn. Bull. U.S.D.A. 1462.
_____ & _____. 1975. Ecology and redescription of the Arizona grape bruchid *Amblycerus vitis* (Coleoptera). Coleop. Bull. 29: 321–331.
_____ & C. N. Slobodchikoff. 1979. Coevolution of *Cassia* (Leguminosae) and its seed beetle predators (Bruchidae). Environ. Entomol. 8: 1069–1064.
Kingsolver, J. M. 1982. Three new species of *Acanthoscelides* (Coleoptera: Bruchidae) from seeds of *Apuleia leiocarpa* (Vogel) Macbride (Leguminosae: Caesalpinioideae) in Brazil. Experientiae (Viçosa) 28: 107–121.
_____. 1984. The Bruchidae associated with seeds of *Parkia* (Leguminosae: Mimosoideae) in northern South America, with descriptions of new species in *Acanthoscelides* and *Mimosestes* (Coleoptera). Entomography 3: 43–73.
Nelson, D. M. & C. D. Johnson. 1983a. Stabilizing selection on seed size in *Astragalus* (Leguminosae) due to differential predation and differential germination. J. Kansas Entomol. Soc. 56: 169–174.
_____ & _____. 1983b. Selenium in *Astragalus* (Leguminosae) seeds and its effects on host preferences of bruchid beetles. J. Kansas Entomol. Soc. 56: 267–272.
Rosenthal, G. A. & E. A. Bell. 1979. Naturally Occurring, Toxic Nonprotein Amino Acids. Pp. 353–385 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores Their Interaction with Secondary Plant Metabolites. Academic Press, London & New York.
_____ & D. H. Janzen. 1983. Arginase and L-canavanine metabolism by the bruchid beetle, *Caryedes brasiliensis*. Entomol. Exp. Appl. 34: 336–337.
_____ & _____. 1985. Ammonia utilization by the bruchid beetle, *Caryedes brasiliensis* [Bruchidae]. J. Chem. Ecol. 11: 539–544.
Southgate, B. J. 1979. Biology of the Bruchidae. Annual Rev. Entomol. 24: 449–473.
_____. 1983a. Handbook on Seed Insects of *Acacia* Species. Food and Agricuture Organization of the United Nations.
_____. 1983b. Manuel de Lutte Contre les Insectes Parasites des Semences D'Acacias. Organisation des Nations Unies pour L'Alimentation et L'Agriculture.
_____. 1983c. Manual sobre Insectos que Atacan a las Semillas de *Acacia*. Organizacion de las Naciones Unidas para la Agricultura y la Alimentacion.

CHEMICAL INTERACTIONS BETWEEN BRUCHIDS AND LEGUMES

A. N. E. Birch[1], M. S. J. Simmonds[2a] and W. M. Blaney[3a]

Abstract

An extensive review of recent literature on bruchid–legume interactions highlights some of the principles associated with host finding by bruchids and the suitability of legume hosts for bruchid development. Chemical and physical factors influencing habitat selection, host finding, host recognition, host acceptance, and host suitability are examined in detail, including aspects of plant biology militating against penetration of the pod and testa by bruchid larvae. Also examined are behavioral and metabolic adaptations of bruchids enabling them to circumvent the plant defenses. The need for further experimental work and field studies is identified. Future investigations should include: behavioral and developmental studies to identify vulnerable aspects of the bruchid–legume interaction; the search for novel allelochemics and for the occurrence of multifactor protection; and investigation of wild relatives of crop legumes for protective traits that may be incorporated into crop varieties by selective breeding or genetic engineering and that are compatible with crop status.

Introduction

Bruchidae are seed-feeding beetles of which a large proportion (84%) are predators of legume seeds (Johnson, 1970). Some are generalists, exploiting many species of legumes, while others are or appear to be specialists having a much more restricted range of hosts. Many of the bruchid genera feed on seeds from more than one legume subfamily or tribe. Thus, 50% of the 56 bruchid genera are able to develop on the Caesalpinioideae, 56.2% on the Mimosoideae, and 59.3% on the Papilionoideae. In addition, 25% of the bruchid genera have been recorded from all three subfamilies of legumes (Johnson, 1981).

In this paper we hope to clarify some of the principles associated with bruchid host finding and host suitability and to provide examples of the role that chemical and physical factors play in mediating these processes. The failure of a bruchid to establish itself on a legume can be the consequence of factors operating at any one of five stages of its life cycle: habitat selection, host finding, host recognition, host acceptance, and host suitability (Fig. 1).

Life Cycle

The female bruchid lays her eggs on or near individual seeds or seed pods. If the eggs are laid on seeds, the larvae burrow down through the egg shell and seed testa into the cotyledons. If eggs are not laid directly on seeds, the young larvae that emerge are able to locate and penetrate the pods and seeds of potential hosts. The entire larval period is spent within the seed if it is large enough to sustain full larval growth. When the seeds are smaller, several may be invaded by the developing larva. Conversely, some seeds are large enough to support the growth of several larvae. Pupation occurs within the seed, and the young adult bites through the testa to emerge.

[1]Zoology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, U.K.
[a]Behavioural Entomology Group at:
[2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3DS, England.
[3]Department of Biology, Birkbeck College, Malet Street, London WC1E 7HX, England.

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Monogr. Syst. Bot. Missouri Bot. Gard. 29:781–809. 1989.

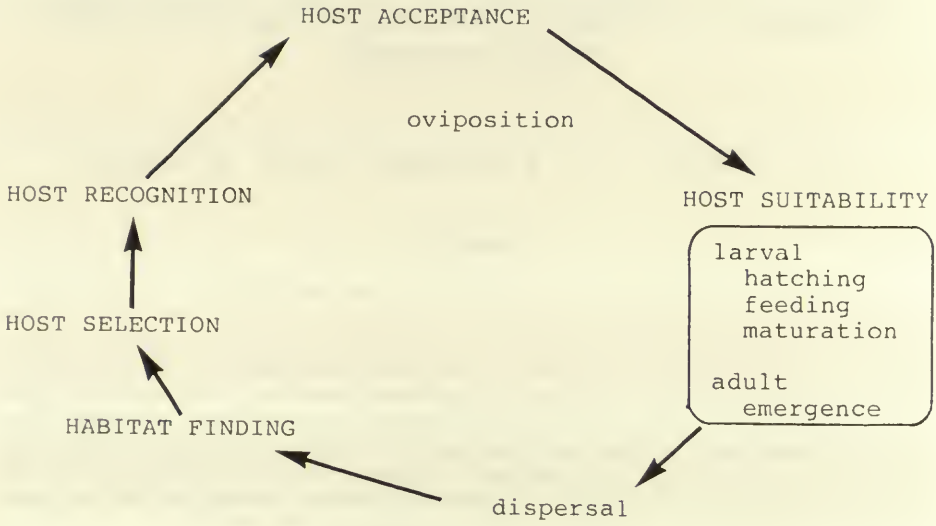


FIGURE 1. Stages in the bruchid–legume interaction.

Of the 1,300 or more species of bruchids, some 12 species have evolved the habit of laying their eggs and completing their life cycle in the stores of mature seeds harvested by man. These pest bruchids occur throughout the Old and New World tropics and to a lesser extent in temperate North America and Europe. The result of this infestation is to reduce the quantity and quality of food available for human consumption and to reduce seriously the germination viability of the seed. In Nigeria up to 100% of the staple crop, the cowpea *(Vigna unguiculata),* can be destroyed during six months of storage (Booker, 1967).

Johnson (1981) has taken account of the ecological, behavioral, and evolutionary aspects of the interaction between bruchids and legumes in proposing a classification of the bruchids into feeding guilds. According to this classification bruchids in guild A lay their eggs on mature or immature pods while the pods are still on the plant. Those in guild B lay on mature seeds after the pods have partially dehisced on the plant. Members of guild C lay on mature seeds lying free on the substrate. By the same criteria, the legumes may be ascribed to three guilds: 1) those with dehiscent pods; 2) those with indehiscent pods; 3) those with partially dehiscent pods. There is not, of course, exclusivity between the guilds of bruchids and those of legumes. Some bruchids of guild A use pods of all three guilds of legumes. Many bruchids are able to discriminate between pods of different ages. Generally the eggs are cemented onto the pod and the larvae burrow into the seed. Many bruchids in guild B are able to utilize a wide range of legumes as long as mature seeds are available. *Stator limbatus* and *S. pruininus* are able to use seeds of 30–40 different host species. By contrast, in guild C, where scattered seeds are utilized, some very specific bruchid–legume associations exist. In some cases it is necessary for the seeds to have passed through the digestive system of ruminants so that the pod valves have rotted away. This occurs, for example, with *S. vachelliae* using the seeds of indehiscent pods of acacias.

SPECIFICITY

Attempts at assessing the degree of specificity of bruchid–legume associations are limited by the available information and reflect the fact that very few studies have been made and only in some parts of the world. Further studies in the tropics will without doubt increase the number of host

records, but we cannot predict whether they will reveal the bruchids to be mainly specialists or generalists. With specialist relationships we need to ask whether they are taxonomic or functional, that is, whether the bruchids are restricted to a species or genus of host plants or to a guild of seeds, perhaps characterized by some common morphological or chemical feature.

The most heavily exploited legumes are the genera *Mimosa* (39 species) and *Astragalus* (32 species), which are attacked by species of *Acanthoscelides* (Johnson, 1981). Sixteen species feed on *Mimosa* and seven on *Astragalus*. It seems that few bruchid species are able to live on many different species of legumes. Johnson's (1981) records show that 82.5% of the bruchids attacking *Cassia* were able to develop in 1–3 species, and only 16.5% were recorded from 5–10 species.

Most work on bruchids has been restricted to the 12 species that are pests of crop legumes. They include the Old World genera *Bruchus*, *Callosobruchus*, and *Carydedon* and the New World genera *Acanthoscelides* and *Zabrotes*. These are only a very small proportion of the bruchid species of the world and arguably are unrepresentative in that they have adapted their life cycle to exploit a man-made environmental niche. Domestication and selection of crop legumes has resulted in the production of larger plants with larger seeds in indehiscent pods and the loss or reduction of natural defensive chemicals. Changes have occurred too in the life cycle of crop legumes. Compared with wild legumes, crop legumes have been selected for decreased seed dormancy and either uniform or differing maturation times. In arid regions, the rapid, simultaneous maturation of seeds is favored, whereas in the moist tropics, where loss during seed storage is high, a longer sequential maturation period is beneficial. Similar changes may occur in the wild plant species, and in both cases there may be concomitant changes in bruchid ecology.

Geographical variations in host plant preferences are known and must be taken into account when classifying the bruchid–legume relationship. Within some bruchid families there are distinct differences in the species of host plants of those members occurring in the Old World and those from the New World, especially between species occurring in South America and those in Africa.

Janzen (1969) has suggested that legumes use 31 traits to counter bruchids, including physical and chemical factors. The interface between the bruchid and the legume can be examined at many levels, but the majority of laboratory studies so far have concentrated on the chemical defenses of the seed. These studies are usually centered on the ability of the larvae to develop within the seed, and attempts are made to correlate this with an analysis of the chemicals contained in the seed. The corresponding field data are based on records of the numbers and species of bruchids emerging from seeds collected in the field. The data suggest that there is a direct relationship between the numbers of adults reared in this way and host selection exercised by the whole population in the field. However, failure to find a bruchid emerging from a pod does not guarantee that the adult did not oviposit on that pod. Finding a bruchid in a pod indicates host suitability but not host preference. To date, the information on host suitability, which has often been used to infer host preference, has been gained from records of the number and species of seeds supporting a particular species of bruchid. The terms "host suitability" and "host preference" should be treated with care, as the latter implies that a bruchid, when confronted with two or more potential hosts, is able to assess their relative suitability before ovipositing.

HABITAT SELECTION

The term habitat can be used to describe a site in which hosts may exist in a form suitable for exploitation by a foraging insect.

The extent to which habitat selection is an important factor in the lives of bruchids varies widely, depending on their life styles and locations, and on variable environmental factors. Bruchids colonizing food stores will generally emerge as adults in a habitat that is perfectly adequate and acceptable for oviposition. The same might apply to individuals of some generations in field conditions where the legume host species is abundant and bears susceptible seeds or fruits contin-

uously for several months at a time (Janzen, 1980). On the other hand, it commonly occurs that individuals of successive generations are obliged to leave the habitat occupied by their parents because suitable hosts are no longer present or, even in grain stores with abundant hosts, because other environmental factors, such as the effects of crowding, promote dispersal activity.

A major cause of unsuitability of a habitat is the temporary absence of host plants at a stage appropriate for predation. Suitable habitats may be separated from each other spatially and temporally, for example, by the maturation cycle of the plants, rendering them unsuitable seasonally. In these two cases, different strategies for survival are appropriate. When suitable habitats are separated by distance, spatial migration occurs in which the insects must perceive and respond to cues that initiate departure, sustain flight in an appropriate direction, terminate flight at a distant location, and identify the suitability of the new habitat. However, when habitats are separated temporally, a period of diapause, or at least reduced activity, may be initiated by certain environmental cues and terminated by others. In both cases a complex sequence of stimuli and responses may be involved, about which we have relatively little information on bruchids. Consequently, in discussing the mechanisms involved we are obliged to refer to other, more widely studied, insect groups and in so doing, to identify areas for future study of the bruchid–legume interaction.

POLYMORPHISM

Some species of insects undergo an obligatory migratory phase in every generation; in others it is facultative, and then the physical capacity for flight may vary. The most obvious manifestation of this is the development of full-sized, functional wings in the adults of a "dispersal" generation while the nondispersing generations are sedentary, having short wings or no wings at all. Such polymorphism is widespread and well documented in aphids (Dixon, 1985) but does occur in other insect orders and has recently been investigated in the bruchid *Callosobruchus maculatus* (Messina & Renwick, 1985). *C. maculatus* attacks the seeds of wild and cultivated legumes, especially *Vigna unguiculata,* and occurs in field conditions and in seed stores. It was noted (Caswell, 1960) that in crowded laboratory colonies the normal sedentary morph, capable of only short flights, was sometimes replaced by morphologically and physiologically different adults capable of sustained flight. Crowding was identified (Utida, 1972) as the most important factor influencing morph determination. Messina & Renwick (1985) showed that the influence of crowding was manifested to different extents in three geographically separate strains, showing that a genetic factor is involved in the ability to respond to the cue. Prevett (1961) has shown that the role of the more active morph is to allow the beetle to leave crowded stores and infest cowpeas in the field.

DISPERSAL

Given that flight capacity has been developed, the insect must then respond to a complex series of cues to achieve dispersal. The beetle may be on the forest floor or low in the vegetation and will respond with negative geotaxis, crawling upwards in the vegetation until a high point is reached from which takeoff is likely to result in successful free flight. This flight will only be initiated and sustained in conditions of low wind speed that allow the insect control over its flights. Sustained flight clear of terrestrial obstructions is facilitated by a positive phototactic response; the insect tends to fly upwards. This response may have a spectral component, as in aphids (see Simmonds et al., this volume).

In long-range migratory flights insects are often carried downwind, but in many cases, especially where relatively short, low flights are made, the insect flies upwind, i.e., showing positive anemotaxis. This phenomenon is well known in male moths flying upwind towards a pheromone

source, such as an emitting female of the same species. The same phenomenon occurs, with host plant odors as the cue, in flying insects (Visser, 1986; Mulkern, 1969) and in others walking over the substrate (Kennedy & Moorhouse, 1969). This phenomenon almost certainly occurs in bruchids moving within a habitat or between habitats, although it has not yet been widely or rigorously investigated. Jarry (1981) documented an invasion by *Acanthoscelides obtectus* of 57 out of 58 plants of *Phaseolus vulgaris* isolated in a cornfield in southern France when the nearest source of the insects was 500 m away. Pouzat (1978), working with a domestic strain of the same insect, showed that females responded to plant odor presented in an olfactometer, and that the response varied with the age of the insects and thus with their physiological state. Olfactory cues signalling a suitable habitat may also eminate from conspecific bruchids already present. Pouzat (1978) found that virgin females of *Acanthoscelides obtectus* would walk upwind in an odorless airstream but that this response disappeared after four or five days. However, if the females were then subjected to male odor their anemotactic response was restored.

Migratory movement should be terminated when a suitable habitat is encountered. The precision of this will vary with the mode of locomotion and its control, and with the state of the potential host vegetation. Termination of flight may be associated with physiological changes in the insect that affect flight directly, such as depletion of food reserves. Alternatively, as in aphids (Kennedy et al., 1961), there may be a change in spectral sensitivity, such that towards the end of a flight, the shortwave (blue) radiation of the sky is less attractive than the longer-wave (green/brown) radiation of the earth. The final stage of the insects' flight may be orientated towards a plant. The ease with which a plant or specific part of a plant may be disocvered by a potential predator is determined partly by factors contributing to the apparency of the plant (Feeny, 1976). The term "apparency" encompasses such factors as the plant's size, shape, persistence, and relative abundance in the community, and the numbers of predatory insects and their host-locating ability.

Another variable is the physiological and behavioral state of the insect. A habitat may be appropriate for an insect for different reasons at different times. Bruchids aggregate in a habitat for mating, possibly in response to visual and chemical stimuli from conspecifics. Some bruchids that feed on nectar or pollen may be attracted to a habitat containing host plants before these plants have any fruits or seeds on which they may oviposit. Many bruchids must feed on pollen before oogenesis and mating will occur. Adults of the pea bruchid, *Bruchus pisorum*, will feed on pollen from many different plant families, but for successful mating they require pea pollen (Pajni & Sood, 1975). Other bruchids, such as *Bruchidius angustifrons*, *Bruchidius pygomaculatus*, *Bruchidius minosae* (Pajni, 1981), and *Acanthoscelides obtectus* (Leroi et al., 1984) feed on pollen, but it is not a requirement for mating. Often members of a bruchid species will aggregate on a host or within a habitat as a result of a high frequency of landings on acceptable, pollen-bearing plants, and this aggregation then results in mating taking place. When habitats are temporally discontinuous, cues from the host species in the habitat may be important in altering the physiological state of the insect. For example, in the dry season the reproductive diapause of *B. atrolineatus* is broken in both sexes by the presence of inflorescences of *Vigna unguiculata* with the result that full ovarian development has occurred by the time the pods are available for oviposition (Germain et al., 1985).

Host Finding

The distinction between habitat selection and host finding is not always clear. In some cases, for example with stored-product pests, once the insect has found the appropriate habitat, it will have found the host as well. Similarly, in a habitat consisting of a legume crop monoculture, there may be no or very few other species of plants present except the host. Alternatively, an insect may have to move extensively within a habitat to locate a host or, having found a host plant, it may have to search further to find the part of the plant appropriate to its needs at that time.

HOST DIVERSITY

The ability of a bruchid to find a host within a habitat depends partly on its host range, that is, whether it is a generalist or a specialist, and partly on the density and diversity of potential hosts within the habitat. The "resource concentration" hypothesis (Root, 1973) suggests that a specialized herbivore would be more likely to discover and exploit hosts occurring in high densities.

The fact that in areas of high plant diversity a single species or a few species of plants may suffer high levels of predation could result from the insects initially being attracted into the habitat by chemical or visual stimuli from nonhosts and then, once in the habitat, responding to specific host stimuli. On the other hand, it may be that chemicals released from nonhosts in a diverse habitat protect potential hosts by repelling or confusing the specialized predator (Stanton, 1983).

GEOGRAPHICAL VARIATION

Another important factor influencing host finding is geographical variation within bruchid species and their legume hosts. This may result in the establishment of relatively localized preferences. Additionally, switches in preference can occur when the bruchid, for whatever reason, develops for as little as one generation in a novel host. For example, Wasserman & Futuyma (1981) have shown that exposure of a biotype of *Callosobruchus maculatus,* normally reared on adzuki bean *(Vigna angularis),* to seeds of pigeon pea *(Cajanus cajan)* resulted in those bruchids emerging from the pigeon pea seeds having an oviposition preference for pigeon peas over the adzuki bean. This type of behavior change could be important in areas where new varieties or new crop relations are introduced. The degree of plasticity in the bruchid's selection behavior could determine the vulnerability of the new plant regime.

Legumes are sometimes cultivated in areas where wild populations of the same plants occur, and this has an influence on bruchid ecology and host-finding behavior. In parts of Mexico, wild populations of *Phaseolus vulgaris* and *P. coccineus* produce ripe pods and seeds briefly during the dry season. With the benefit of irrigation and storage of seeds, cultivated selections of the same plants are grown throughout the year. These two different cycles of reproduction in the plant have been exploited by different populations of *Acanthoscelides obtectus.* The wild plants are attacked by a univoltine population of *A. obtectus* which enters imaginal diapause until ripe pods are available again. During this diapause, adults remain in the dry, curled-up *Phaseolus* leaves that have remained on the plant (Biermont & Bonet, 1981). This behavior occurs in other diapausing bruchids (De Luca, 1973; Prevett, 1967; Pajni & Sood, 1975). The cultivated *Phaseolus* plants are attacked by a multivoltine population of *A. obtectus.* The diapause in the univoltine population has presumably developed as an adaptive function synchronizing the life cycle of the bruchid with that of its host plant. With the introduction of the cultivated plants, this diapause is no longer necessary.

INSECT RESPONSES

Detailed information on the activities of an individual bruchid finding a host within a habitat is generally lacking. When at a distance from its host, the bruchid, by analogy with other species, probably responds to visual and/or olfactory cues. It would be useful to know whether bruchids alight on hosts within a habitat in relation to a plant's abundance, or whether some degree of selection has occurred before landing.

More information is available on stored-product pests. Our unpublished experiments suggest that they move at random within the environment. Chemical cues are apparently encountered on contact with or in the immediate vicinity of a seed (Simmonds & Blaney, unpublished). When this stimulus is encountered locomotion stops or is reduced and the bruchid begins to examine its immediate

environment closely. If the arrestment is not sustained, the bruchid will reject the area and resume random movements. Another indication of response to some perhaps invisible stimulus is a sudden increase in the rate of turning of the walking bruchid. This turning behavior retains the insect in the immediate vicinity of the cue. We have monitored such behavior in experiments in which the bruchids were able to pass over seeds but were not able to contact them, since a fine mesh screen separated the bruchids from the seeds. Turning behavior was observed more often with potentially acceptable host seeds than with nonhost seeds. This shows that bruchids can discriminate between volatile chemicals from seeds of different plants, but we need to know much more about the nature of the stimuli that influence pre-alighting and post-alighting behavior and whether these stimuli are effective if perceived in a random manner.

Host Recognition

Once on the plant, the bruchid is exposed to a complex array of chemical and physical stimuli that are sensed by various peripheral receptors. If the correct stimuli are received, they will initiate feeding or oviposition. The details of this stage in the insect–plant interaction are only poorly understood, and most information available concerns oviposition.

We know that chemical and physical stimuli are important and that the precise stimuli to which the bruchid responds will depend in part on its feeding guild. The pod and seed surfaces are thought to have an array of chemicals that influence bruchid behavior, deterring or stimulating oviposition, but surprisingly little is known about them. Our experiments and the work of other authors (Honda et al., 1976) suggest fatty acids are possibly important as chemical cues.

If the female bruchid oviposits on pods, she will need to be able to assess the quality of the pod. As pods mature their chemical and physical characteristics change markedly (Messina, 1984). Immature, green pods are likely to produce more and different volatiles than older, dry pods. The seeds likewise vary in physical and chemical characteristics in ways that may or may not affect their suitability for larval development.

Insect Behavior

To characterize the important chemicals that influence host recognition behavior we need to identify the elements of the relevant behavior and make carefully observed and quantified experiments on the effects of chemicals on it. Such experiments should assess whether the importance lies in a single chemical or in a mixture, whether the chemicals are volatile or perceived only by surface contact, and whether the physiological state of the bruchid influences its response to the chemicals.

Oviposition behavior begins with the bruchid walking over the surface of the pod or seed having apparently only the tarsi making contact with it. Then the antennae and mouthparts are lowered and wiped over the surface of the seed or pod. During this examination the sensilla on the bruchid's antennae or mouthparts encounter chemicals that exert either a positive or negative effect on reproductive activity and oviposition behavior (Pimbert & Pierre, 1983). It would be of value to know whether host recognition by a given insect is induced by positive stimuli exclusively or whether positive and negative stimuli are balanced. By analogy with locusts and lepidopterous larvae (Blaney, 1980; Simmonds & Blaney, 1984), the capacity to make decisions based on the assessment of conflicting stimuli may vary between specialist and generalist bruchids, the latter being able to make more complex assessments.

Identification of the sensilla involved in detecting chemical cues allows the putative chemicals to be screened in electrophysiological tests against the sensilla (Blaney et al., 1987), providing a further test of the identification of relevant chemicals. By observing the bruchid examining host and

nonhost pods and seeds and by analyzing the behavioral responses in detail, we refine our knowledge of host selection.

Little information of this sort is currently available in the literature. The host records in such papers as those by Janzen (1980) and Johnson (1981) are based on records of adult emergence, which reflect host suitability. More data are desirable concerning the oviposition behavior of the bruchids to determine whether they restrict their foraging activity to legumes from which their progeny subsequently emerge, or whether they sometimes oviposit on others unsuitable for their larvae. Some of our laboratory experiments show that bruchids will oviposit readily on seeds that are not normally attacked under field conditions. We have filled seed-sized gelatin capsules with ground 'flour' from resistant cultivars of chickpea and find that eggs laid by *Callosobruchus maculatus* on the capsules result in full larval development, although eggs would not normally be laid on the intact seeds. Thus, two important questions of field biology whose answers would provide insight into the evolution of bruchid–legume interactions remain unanswered. Are host recognition and oviposition highly correlated with hosts potentially suitable for larval development? On the other hand, do bruchids avoid oviposition on legumes that could in fact support their development?

Host Acceptance

It is a common feature of insect–host plant interactions in relation to feeding (Blaney et al., 1985) and to oviposition that an insect in contact with its normal host will, on inspection, reject that host. The reasons for this may be numerous and are not readily accessible to the observer, as they presumably depend upon features of the state of the insect and of the plant that are difficult to measure and whose relative importance is difficult to assess.

The volatiles that make up the "essential oils" of the plant may be important in mediating not only host location but also, at closer range, host acceptance. Additionally, less-volatile chemicals may act as contact stimuli. A study that distinguished chemicals available on the surface of pods and seeds from exclusively internal chemicals could do much to resolve many of the outstanding questions of legume–bruchid interactions.

Oviposition Behavior

The time taken and behavioral sequences involved in host examination vary among and within bruchid species depending on the physiological state of the female and the host it is examining. There are very few studies on the oviposition behavior of bruchids; the best-documented are those of Pouzat (1978) and Wasserman (1981). Zachariae (1958) observed that on *Phaseolus vulgaris*, the female of *Acanthoscelides obtectus* walks up and down the ventral suture of the pod. She then penetrates the surface with her mandibles and lays 1–20 eggs in the depression so formed. Before oviposition, the ovipositor, with its array of tactile and chemosensory receptors, is rubbed over the surface. During this examination the bruchid will receive information on the topography of the surface and on its moisture and chemical content. This information is used in assessing the acceptability of the pod.

Many bruchids, including species of *Zabrotes*, *Bruchus*, and *Callosobruchus*, cement their eggs to the substrate and leave a secretion or epideictic pheromone on and around the egg. This marker pheromone signals to arriving conspecifics that the host already bears eggs; on encountering the pheromone, the newcomers usually disperse to a different host. As the number of seeds without eggs decreases, the effectiveness of the epideictic pheromone decreases.Oshima et al. (1973) showed the epideictic pheromone of *Callosobruchus chinensis* to contain monoterpenoids, which are also present in pod exudates and on the surface of legume pods and seeds, and could therefore be important in host acceptance. We have tested the responses of *C. maculatus* and *Zabrotes subfas-*

ciatus to a set of monoterpenoids that have been recorded as fragrances from legume flowers. Some of these compounds, such as limonene and linalool, have been shown to occur in the epideictic pheromone of *C. chinensis*, where they act as an oviposition deterrent. As the female encounters these compounds while searching for oviposition sites, she moves away, thus rejecting the seed for further oviposition (Oshima et al., 1973). Many sesquiterpenes occur in legume pods, but little has been done to see if they have an ecological role. Sesquiterpenes from other plant families, such as Canellaceae, act as potent deterrents to insects (e.g., warburganal from the genus *Warburgia*, [Ma, 1977]).

EPIDEICTIC PHEROMONES

Our group is investigating epideictic pheromones of bruchids and surface extracts from rejected and accepted hosts. To evaluate the biological activity of these we derive quantitative data from behavioral assays; we then attempt to correlate these data with sensory responses to the compounds obtained by electrophysiological recording from the sensilla on the antennae, and maxillary and labial palps of the bruchids. The electrophysiological assay is a powerful tool in testing for and identifying the important biologically active ingredients in markers and in attempting to correlate their effects with those of the natural compounds that inhibit oviposition on the surface of pods and seeds.

We have shown that for *Callosobruchus maculatus* and *C. chinensis* oviposition decreases as the level of their epideictic pheromone on the seeds increases, but it has not yet been possible to estimate the quantity of marker left by a single female. However, we have treated glass balls with an extract that stimulates oviposition and allowed bruchids to lay a known number of eggs on the balls. When these balls, and others with the stimulant only, are then exposed to fresh bruchids and the behavior on the two sets of balls is monitored, it is possible to assess the effectiveness of the marker pheromone in preventing further oviposition.

Another factor critical to the outcome of host acceptance, i.e., oviposition, is the time spent by the bruchid in "handling" (examining) a potential host. The reasons why a bruchid spends more time handling one type of host than another relate to physical and chemical stimuli associated with the host and to facets of the bruchid's physiology at the time.

Host Suitability

Selection of a potential host plant by the adult female for oviposition does not guarantee suitability of the plant for larval development. An initial impediment to larval development may be the difficulty encountered by the neonate larvae in transferring from the egg to the plant tissue. Depending on whether a bruchid oviposits on pods, on seeds inside pods, or on seeds after dispersal, the hatched larvae must penetrate the pod wall, the testa, or both in order to reach their ultimate food source (cotyledons, endosperm, embryo) inside the seed (Johnson, 1981). The pod wall and testa can act as mechanical and/or chemical barriers to newly hatched larvae, as well as to older larvae of species that feed on multiple seeds. Plant characters likely to be involved in these defense mechanisms include tissue hardness, thickness, and chemical content (Janzen, 1977b). There are likely to be interactions between mechanical and chemical properties of these tissues that could maximize their defensive value. The precise nature of allelochemicals involved in pod and testa defense is largely unknown due to a general ignorance of the chemical constituents, as is their localized concentrations and their importance in the ecology of bruchid–legume interactions. As plants have limited resources for defense, they are likely to have evolved an order of priority for protection that ensures that organs or tissues essential for future regeneration, such as the embryo, are well protected (Rhoades, 1979). Plants have probably also been subject to strong selection pressure for the ability to reutilize resources invested in defense by later translocating these chem-

icals in some form to the seedling for defensive or nutritional purposes (Janzen, 1976a). Such a strategy would be more difficult and costly to operate on defensive compounds stored in the pod or testa than those in the seed (McKey, 1979).

SEED DISPERSAL PATTERNS

Legumes can be ascribed broadly to three different seed dispersal categories, depending on whether pods are dehiscent, indehiscent, or partially dehiscent (see the section above and Augspurger, this volume). These categories are determined partly by interactions between the chemical and physical properties of the pod wall and the environment. It is likely that the different dispersal strategies have also evolved as a result of selective pressure to escape from seed predators. Bruchids have in turn evolved specific oviposition strategies by attacking seeds at different times and in different ways (Johnson, 1981). These behavioral patterns, synchronized to physical and chemical changes during pod and seed maturation, determine the preferred site and time of oviposition and hence the chemophysical properties of the pod or testa at the time of larval penetration. As described above, Johnson (1981) divided bruchids into three guilds, the first ovipositing only on pods, the second only on seeds within pods, and the third only on seeds after dispersal. Some pest bruchids (e.g., *Callosobruchus maculatus*) may be placed into a fourth guild, since some have evolved the ability to oviposit on pods and seeds (Alzouma, 1981). Johnson (1981) found pod dehiscence (e.g., *Leucaena, Mimosa, Canavalia* spp.) to be the most effective of the three seed-dispersal patterns in reducing seed predation. Providing seeds were well scattered, attack from nearly all bruchids in the first two guilds and most in the third was avoided. At least one component of this escape mechanism is that it protects seeds from larvae in the first guild, developing from eggs laid on pods, by scattering the seeds before the larvae reach them (Cushman, 1911). Legumes with indehiscent pods (e.g., some *Vigna unguiculata* genotypes; Caswell, 1984) not only prevent second-guild bruchids from ovipositing on their preferred site, seeds inside the pod, but may also use the pod wall as an extra barrier to larvae hatching from eggs laid on the outside surface of the fruit. In addition, any gap between the inner pod wall and the seed inside may increase larval mortality. For example, successful seed penetration by *C. maculatus* larvae, laid on the pods of *V. unguiculata*, requires the seed to be directly under the oviposition site and to be firmly appressed to the inner pod wall (Messina, 1984; Pate, this volume). Similarly, directed boring behavior occurs in first instar larvae of *Acanthoscelides obtectus* that hatch from eggs laid within the pod cavity of *Phaseolus vulgaris*. They are stimulated to bore into the testa by a thigmotactic response produced by a contact area between two seeds or between a seed and the pod wall (Thiery & Jarry, 1985).

TISSUE HARDNESS AND THICKNESS

Several studies have implicated pod walls (e.g., Fatunla & Badura, 1983; Serrano et al., 1983; Schoonhoven et al., 1983; Caswell, 1984) or testas (e.g., Janzen, 1977b; Messina, 1984) of wild and cultivated legumes as mechanical barriers to larval penetration. This defense appears in some cases to be associated with thickness of the non-nutritious pod wall (Akingbohungbe, 1976) or testa (Podoler & Applebaum, 1968), probably by causing starvation of neonate larvae before seed penetration is complete. In some other cases where those tissues are not abnormally thick, failure of penetration appears to be due to their degree of hardness. In studies on testas of *Phaseolus vulgaris* seeds in which hardness was quantified, Thiery (1982) demonstrated that this property directly reduced frequency of penetration and was influenced independently by moisture content and age of seed. In further studies on a wide range of testas of Leguminosae, Thiery (1984) found that in some seeds failure of penetration appeared to be influenced by additional factors. These include the ligneous texture and structural arrangement of cells within the epidermis and subepi-

dermis of the testa. Sections through larval penetration holes in testas of several genotypes of cowpea revealed that larval mandibles act with a transverse chipping motion. There is less resistance to this type of mandible movement in genotypes having more loosely packed macrosclereids in the subepidermis than in those with tightly compressed helical cells in this region of the testa (Nwanze & Horber, 1976). The high rate of larval mortality during penetration of many legume pod walls or testas is therefore due at least in part to stresses exerted on the individual by combined effects of tissue thickness, hardness, and cellular structure.

REDUCED EGG ADHESION

Many bruchids glue their eggs firmly to the pod wall or seed surface with a sticky, rapidly hardening mucuslike substance secreted after oviposition. In *Bruchidius atrolineatus*, which attaches its eggs onto pods of cowpea, this mucus enters the pod stomata before hardening and provides a firm anchorage for the egg on the pod wall (Biermont et al., 1982) and possibly gives neonate larvae a firm base from which to exert pressure against the pod or seed in order to initiate penetration (Nwanze & Horber, 1976). Seed penetration by *Callosobruchus maculatus*, which glues its eggs to the seed surface, depends on force exerted by the mandibles. This force can only be applied if there is sufficient leverage on the pronotal chitinous plate by downward abdominal thrusts.

Plant characteristics (mechanical and chemical) that reduce the ability of bruchids to glue their eggs to the plant surface probably evolved as a defense against insect penetration. The pods of several thorn acacias have flaking surfaces that can be shed, together with eggs and hatched larvae, before complete penetration to the seed is possible (Janzen, 1969). Some legume pods are free from cracks, thus reducing suitable oviposition sites for bruchids, such as *Bruchus prosopis*, which require a rough surface for firm egg attachment (Bridwell, 1918). The production of gums by pods of species of *Prosopis*, *Acacia*, and *Cassia* following initial larval penetration pushes off unhatched eggs and obstructs penetration by larvae (Bridwell, 1920; Hinckley, 1960). The surface of some seeds, for example *Bauhinia monandra*, swell on pod opening and similarly detach eggs and prevent larval penetration (Bridwell, 1918). Rough testa surface textures due to morphological features, including pits, ridges, or spines, may serve a dual role in seed protection against bruchid attack. They can reduce oviposition and can prevent attachment of eggs to the seed surface. Increased larval mortality of *Callosobruchus maculatus* has been attributed to poor egg adhesion on the pitted surface of rough-coated cowpeas (Nwanze & Horber, 1976). Very smooth waxy cutin surfaces can similarly prevent *C. maculatus* eggs adhering to seeds of some wild *Vigna* species (Birch, unpublished).

TOXIC SURFACE CHEMICALS

Pods of several legume species are covered with glands that secrete chemicals onto the fruit surface and sometimes give protection from insect attack. Glands on pods of *Amorpha fruticosa* secrete a toxic compound called "amorpha," which acts as a contact and stomach insecticide (Brett, 1946). Legumes *Parryella filifolia* and *Errazurizia rotundata*, two plants closely related to *A. fruitcosa*, also have pod glands producing similar aromatic compounds with reported insecticidal properties (Kearney & Peebles, 1960). Pods of the cultivated chickpea, *Cicer arietinum*, are reportedly free from bruchid attack in the field, although seeds in storage are readily attacked. The pods of this plant are covered with glands that secrete an exudate rich in malic acid. Laboratory tests exposing mature pods to bruchids have shown that, although a few eggs are laid, the seeds remain free of larvae (Reed et al., 1980). Malic acid or other components of the glandular secretion may act as a bruchid larvicide and have been implicated in chickpea resistance to species of *Heliothis* (Rembold, 1981).

The outsides of legume seeds are covered in a waxy, fatty, hemicellulose or pectinaceous layer termed cutin (Gunn, 1981). This is deposited in a continuous but variable layer over the external surface of the testa. Experimental exposures of bruchids to nonhost seeds (Janzen, 1977b; Johnson, 1981) indicate that seeds of several legumes, including species from *Acacia, Mucuna, Ormosia, Cassia, Canavalia,* and *Parkinsonia,* appear to have potent insecticidal, repellent, or antifeedant compounds in this cutin layer. These chemicals can kill larvae or prevent them from feeding while still outside the seed, after no or minimal (0–0.1 mm) testa penetration. Although positive chemical stimuli influencing larval penetration are thought to be present on some host seed surfaces (Thiery, 1982), it is unlikely that failure to commence penetration is caused by a lack of them; neonate larvae can "mine" up to 0.5 mm into inert plastics lacking any phytochemical cues (Janzen, 1977b; Thiery, 1984).

Penetration of *Phaseolus* seeds by larvae of *Acanthoscelides obtectus* has been studied in detail by Leroi & Jarry (1981) and Thiery (1984) who found that the seed testa is an important barrier against larval penetration in all species tested, including one of the normal hosts, *Phaseolus vulgaris*. Larval mortality outside the seed ranged from 60% on seeds of the cultivated host plant, to 100% in wild *Phaseolus* species. Neonate larvae die without any sign of testa penetration when attacking seeds of wild. *P. lunatus* and *P. acutifolius*. This is thought to be due to the presence of unidentified surface chemicals that may suppress biting activity or deter further feeding (Thiery, 1984). Most larvae of *Callosobruchus maculatus* and *C. chinensis* also die inside eggs laid on the testas of wild *P. acutifolius* var. *latifolius* after minimal penetration of a relatively thin testa (Birch et al., unpublished). These unidentified surface chemicals on seeds of wild *Phaseolus* species appear to be effective against several other bruchid species. This phenomenon may occur in other genera of legumes (e.g., Janzen, 1977a, b), although it is apparently lacking in seeds of some domesticated forms (Birch et al., 1985b).

TOXIC INTERNAL CONSTITUENT CHEMICALS OF POD AND TESTA WALLS

There appears to be little literature on the chemical content of legume pods. Of the classes of known antibruchid secondary compounds in legume seeds, we have only found records of trypsin inhibitors in the green pods of the winged bean (*Psophocarpus tetragonolobus;* Tadera et al., 1984) and hydrolysable tannins in pods of several species of *Caesalpinia* (Swain, 1965). There are no field records of winged beans being attacked by bruchids. The exposed seeds are resistant to attack by several pest bruchids (Dobie et al., 1979), and part of this field resistance may be due to the presence of trypsin inhibitors in the pod. This class of chemicals shows activity against *Callosobruchus maculatus* larvae in cowpea seeds (Gatehouse & Boulter, 1983). It is not known how the presence of pod tannins might affect penetrating larvae. At least some insects are insensitive to hydrolyzable tannins, which can cause deleterious effects on midgut epithelium and perhaps other organs outside the gut of susceptible insect species (Bernays, 1981). Similar effects on bruchid larval guts are caused by seed lectins (Gatehouse et al., 1984).

Bioassays monitoring larval mining behavior by the generalist *Callosobruchus maculatus* on a wide range of nonhost wild legume seeds (Janzen, 1977b) revealed that in several crops a large proportion of larvae die either after some attempt at internal mining (0.1–0.3 mm) or immediately after passing through the seed coat. This indicates the presence of toxic or antifeedant chemicals inside some of these tissues that can kill neonate larvae rapidly or slowly by cummulative action. Attempts to demonstrate the toxicity of testa constituents have been made by incorporating ground-up testa into artificial diets (see Table 1). Winged bean testa did not significantly depress survival of *C. maculatus* below a level occurring on diets incorporating testa of a normal host, cowpea (Dobie et al., 1979). This result contrasts with those from ground testa of *Phaseolus vulgaris* fed to *Acanthoscelides obtectus* (Stamopoulos & Huignard, 1980). When ground testa was added at levels of 10% to the standard diet of ground cotyledon the mortality of larvae was 98% compared

TABLE 1. Types of experiments used to investigate defense mechanisms in the testas of legume seeds.

Experiment	Methodology	Examples
Bioassay of intact plant tissue (nonhost)	Observations on sequence of events and mortality factors from egg hatch to larval death	Janzen (1977) Thiery (1984) Birch et al. (1985b)
Artificial perforation/ removal of tissue	Comparison of larval penetration with and without treatment to pod or testa	Johnson (1981) Leroi & Jarry (1981)
Tissue transfer (post oviposition)	Eggs which have been cemented to nonhost removed intact with piece of test tissue; this transferred and attached to host seed with testa removed	Birch et al. (1985a)
Oral toxicity	Finely ground tissue incorporated into diet contained in artificial seeds inoculated with eggs	Stamopoulos & Huignard (1980)
Chemical investigation	Extraction of separated tissue to determine localized concentrations	Miller (1967)

with 18% with standard diet alone. Although such experiments can demonstrate the potential toxicity of testa constituents, their role as and effective defense mechanism remains largely unproven since many bruchid larvae may mine through the intact testa without ingesting any tissue (Birch et al., unpublished).

There have been few studies of the chemicals of testas, as opposed to the whole seed. There is evidence for the localized accumulation of polyphenolic compounds, including condensed tannins in testas of *Crotalaria* species (Tookey et al., 1963) and *Vicia faba* (Griffiths, 1981). Although quantitative data are generally lacking, it is likely that a diverse range of phenolic compounds are common constituents of many legume seeds (Janzen, 1977b; Asquith & Butler, 1986) and are probably present at relatively high concentrations in testas, compared with levels occurring inside seeds (Waterman, pers. comm.). For example, seeds of two tropical rainforest legumes. *Dalbergia parciflora* and *Intsia palembanica*, contain 8% and 15% condensed tannins respectively in their testas compared with 2.5% and 0% inside their seeds (Waterman, unpublished). Condensed tannins from western hemlock were of relatively low toxicity to the bruchid *Callosobruchus maculatus* compared with other classes of secondary plant compounds tested (Janzen et al., 1977), although qualitative differences in toxicity among different types of legume tannins may exist. Levels of tannins present in the few legume testas investigated are well above the 5% level found to reduce bruchid larval survival. Condensed tannins isolated from the testa of *Vicia faba* were found to be toxic to larvae of *C. maculatus* (Boughdad et al., 1986). Although the role of tannins in plant defenses against insects is a matter of debate (e.g., Bernays, 1978; Martin & Martin, 1984), these phenolic compounds are considered by some authors to be broad-spectrum antinutritional chemicals with antifeedant (Feeny, 1975), digestability-reducing, and enzyme-inhibition activity (Rhoades & Cates, 1976) as well as histopathological activity (Sternly & Berenbaum, 1985).

Alkaloids have been detected in testa tissue of *Crotalaria intermedia* and *C. spectabilis* (Tookey et al., 1963). Of the various secondary plant chemicals tested against *Callosobruchus maculatus* by Janzen et al. (1977), alkaloids were the most toxic, with some effective at a dose of 0.1% concentration in the diet. The high toxicity of alkaloids to at least one pest bruchid suggests that even at low concentrations they may inhibit feeding or kill larvae of nonadapted species.

SPECIFIC DEFENSE CHEMICALS OCCURRING IN COTYLEDONS

Many classes of secondary chemicals in cotyledons have been shown experimentally to have a deleterious effect on the development of bruchid larvae. These are discussed below.

Rotenoids. The insecticidal properties of rotenoids, including rotenone, have long been known to native peoples in various parts of the world. Examples of this type of complex isoflavonoid accumulate in root, leaf, and seed tissues of several legume genera, including *Lonchocarpus, Derris, Tephrosia,* and *Mundulea* (Ingham, 1983). At least 10 rotenoid structures are known to occur in higher plants (Fukami & Nakajima, 1971), of which rotenone was the first to be isolated. Their activity against several insects has been investigated under laboratory conditions. The major effect of rotenoids on insects is to cause a dramatic decrease in oxygen uptake, which results in death. This is due to their potent inhibition of mitochondrial respiratory metabolism. It appears that they inhibit NADH-linked respiration by specifically binding to a component of the respiratory chain between NADH dehydrogenase and coenzyme Q (Yamamoto, 1970). In a comparison of 35 rotenoids for contact toxicity to adult *Callosobruchus chinensis,* rotenone was found to be the most effective, although several other naturally occurring rotenoids showed significant activity (Fukami et al., 1959).

We have isolated and identified four known rotenoids from seeds of the legume *Lonchocarpus salvadorensis,* which appears to be attacked by very few indigenous bruchids in Costa Rica (Janzen, pers. comm.). Laboratory bioassays incorporating these rotenoids at concentrations occurring naturally in seeds into the diet of *Callosobruchus maculatus* larvae demonstrated that rotenone (0.29% in seed) and degeulin (0.22% in seed) totally inhibited larval development. A third rotenoid, elliptone, tested at its naturally occurring concentration of 0.06% was also effective, causing over 60% reduction in larval survival (Birch et al., 1985a). Although *C. maculatus* successfully oviposited on seeds of *L. salvadorensis* and larvae successfully penetrated the testa, they died immediately on entering the cotyledon. A few specialist bruchids do develop in host seeds containing rotenoids, e.g., *Piscidia mollis, Tephrosia candida,* and *Pachyrhizus erosus* (Johnson, 1981; Center & Johnson, 1974; Janzen, 1980), but *L. salvadorensis* seems to be protected effectively from most seed predators.

Polysaccharides. Many species of legume seeds accumulate large quantities of storage polysaccharides. These have been detected and characterized in soybean, lima bean, haricot bean, garden pea, and groundnut (Applebaum et al., 1970). These macromolecules vary in type and quantity among legume species and varieties.

Ishii (1952) was the first to report the possible involvement of polysaccharides in seed incompatability. He attributed the resistance of haricot beans *(Phaseolus vulgaris)* to *Callosobruchus chinensis* to their pentosan content, which was high compared with that in the host, adzuki bean *(Vigna angularis).* Later chemical investigations revealed a specific soluble heteropolysaccharide fraction from *P. vulgaris,* present in the seed at 1%, which increased larval mortality, decreased larval development rate, and decreased adult fecundity (Applebaum et al., 1970). This heteropolysaccharide fraction was found to be composed of galactose, glucose, xylose, arabinose, and traces of rhamnose and differed from many other legume heteropolysaccharides in lacking mannose. Tests revealed that at a concentration of 1% w/w the heteropolysaccharide prevented development of *C. chinensis* but not of *Acanthoscelides obtectus,* for which *P. vulgaris* is a host (Applebaum & Guez, 1972). At a concentration of 2% the polymer partially inhibited development of *A. obtectus,* and reduced adult emergence by nearly 50%. Unlike *A. obtectus, C. chinensis* can only partially digest the basic heteropolysaccharide structure, using its α-glucosidase and α-galactosidase enzymes.

Phaseolus vulgaris also contains about 35% starch, stored in distinct granules, which can be isolated intact. Although this amount is adequate for larval development, some physical character-

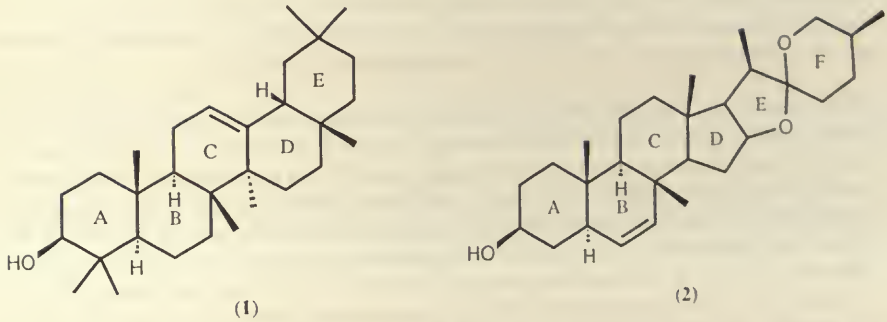


FIGURE 2. The basic structures of triterpenoid and steroid saponins. (1) Triterpenoid sapogenin. (2) Steroid sapogenin.

istic of the granules renders them resistant to the α-amylases of *Callosobruchus chinensis* larvae and therefore unavailable as food (Applebaum et al., 1970). Similarly, starch granules of resistant garden pea varieties are of poor nutritional value to *C. chinensis* (Podoler & Applebaum, 1971). It therefore appears that the heteropolysaccharide and starch stored in *P. vulgaris* seeds are unavailable as carbohydrate sources and this is at least partially responsible for the resistance of these seeds to nonadapted larvae like *C. chinensis*.

Saponins. Saponins are steroid (C_{27}) or triterpenoid (C_{34}) structures (sapogenins) attached to sugar chains of variable length (see Fig. 2) composed of pentoses, hexoses, or uronic acids (Applebaum & Birk, 1979). Saponins have foam-forming properties in aqueous solutions and can cause acute toxicity to man if injected intravenously. Taken orally saponins are more toxic to cold-blooded than warm-blooded animals and have been used traditionally as fish poisons. Saponins have been detected in numerous legume seeds. A survey of major food legumes (Fenwick & Oakenfall, 1983) revealed high levels in chickpeas *(Cicer arietinum)*, soybeans *(Glycine max)*, and varieties of haricot beans *(Phaseolus vulgaris)*. Lower levels were detected in peanut *(Arachis hypogaea)*, winged bean *(Psophocarpus tetragonolobus)*, lentil *(Lens culinaris)*, faba bean *(Vicia faba)*, and lima bean *(Phaseolus lunatus)* seeds.

The widespread occurrence of saponins in seeds of legumes and other plants suggests that they may serve as a chemical defense against insects. Many have a bitter taste that can deter herbivores from feeding. The effect of legume seed saponins on bruchids has been examined in detail using *Callosobruchus chinensis* as a model, since this bruchid is unable to develop in seeds of several saponin-rich legumes, including soybean, navy bean, and peanut (Applebaum et al., 1969). Certain fractions from soybean saponin were found to be highly detrimental to the development of *C. chinensis* larvae, although sapogenins alone or their sugar constituents had no adverse effects. In vitro experiments have shown that saponins from different legume species differ in their resistance to larval hydrolytic enzymes (Applebaum et al., 1965) and that these differences may explain at least part of the host incompatability of certain legume seeds to *C. chinensis*, due to toxicity of the intact saponin molecules.

Several possible mechanisms for the toxicity of intact saponins to nonadapted bruchids have been suggested (Applebaum & Birk, 1972). The failure of fully grown larvae to pupate after feeding on soybean saponins may be due to hormonal imbalance, or they may act by altering membrane permeability in the larval gut. Another possible mechanism may be due to some of these chemicals acting as feeding deterrents. Saponins have been demonstrated to deter feeding by several insects, including the grass grub *Castelytra zealandica* (Sutherland et al., 1975) and aphid *Myzus persicae*

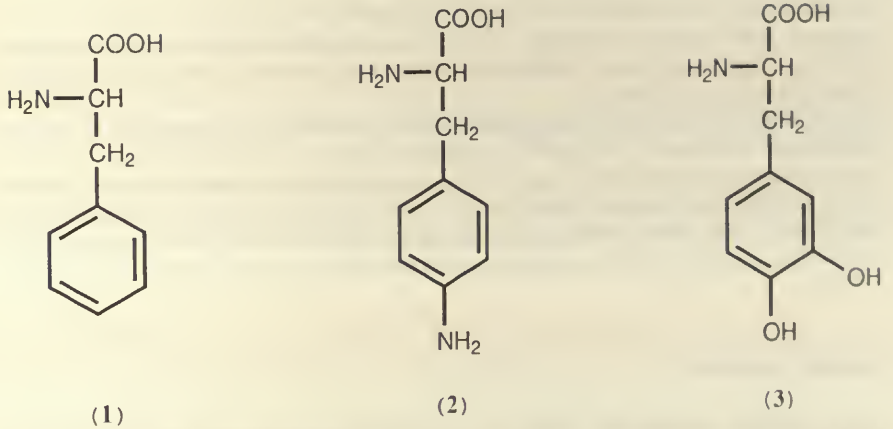
(Applebaum & Birk, 1972). Saponins may also act as nonspecific inhibitors of insect enzymes; for instance, the saponin fraction of soybean was found to be active against α-chymotrypsin, protease, and cholinesterase enzymes of *Tribolium* larvae (Applebaum & Birk, 1979).

Lectins. Lectins are widely distributed in seeds of the Leguminosae—in over 600 species and varieties (Janzen et al., 1986). Most lectins are glycoproteins containing 4–19% carbohydrate covalently bound to proteins. Molecular weights vary from 100,000 to 150,000, and the molecules usually consist of four subunits, which may or may not be identical. A few lectins (e.g., those isolated from lentils and lima beans) are dimers. Each subunit generally has a sugar-binding site, which enables multivalent lectins to bind to and precipitate glycoproteins or large polysaccharide polymers, as typified by lectin agglutination of red blood cells. Some lectins are extremely toxic to mammals, e.g., ricin from castor bean *(Ricinus communis)* and abrin from rosary pea *(Abrus precatorius).* The first report of toxicity to a bruchid, *Callosobruchus maculatus,* by a lectin purified from *Phaseolus vulgaris,* was by Janzen (1976b). In this study the lectin was added to an artificial diet at concentrations between 0.1% and 5%, covering the naturally occuring concentration range of 0.6 to 3.0%. No bruchids survived in a diet of 5% lectin, and even at 0.1% significantly fewer larvae developed into adults. Toxicity due to excess novel protein addition was ruled out by the inactivity of heat-treated lectin, even at 5%. Lectins of *P. vulgaris* are tetrameric molecules containing two types of subunit. Those termed "L type" bind to mammalian lymphocytes while those termed "E type" bind to erythrocytes. The ratio of these subunits, which differs among different *P. vulgaris* genotypes, appears to be important in determining the degree of toxicity. Although studies on rat suggest that both subunit types bind to the gut lumen surface, the E type is thought to be more toxic. In studied on *C. maculatus,* the lectin globulin fraction, which is predominantly E type, was found by Gatehouse et al. (1984) to be more toxic than the predominantly L type albumin fraction.

These preliminary findings suggest that the molecular heterogeneity of different legume lectins is important in the degree of toxicity and probably operates at a species-specific or biotype-specific level for both legume and bruchid involved in the interaction. Some lectins may also act as inhibitors of enzymes, e.g., carbohydrases, esterases, and ATPases, as demonstrated in studies on the rat (Pusztai et al., 1979). However, in preliminary studies on the major digestive enzymes of *Callosobruchus maculatus* no such effects were observed (Gatehouse et al., 1984).

Nonprotein amino acids. Besides the 20 amino acids that are the normal constituents of plant and animal proteins, several hundred nonprotein amino acids have been isolated from plants and microorganisms (Rosenthal & Bell, 1979). Although many resemble protein amino acids in structure, they are not used by the plant as building blocks for proteins. Many may accumulate in legume seeds, sometimes at very high concentration, such as 5–10% dry weight, and usually occur in a free state, as simple peptides. Most have limited distribution within Leguminosae and are often useful chemotaxonomic markers of legume genera, subgenera, or species. Their ecological functions, other than being a reserve of storage nitrogen in the seed, have been investigated, and at least one role of these compounds is to protect seeds from insects and other predators. Comparisons involving seed predation levels and chemistry (Rehr et al., 1973) have revealed that some legume seeds that were relatively free from insect attack contain high concentrations of nonprotein amino acids. For example, *Gymnocladus dioica* and *Albizia julibrissin* were found to be free from endemic seed predators, including bruchids, and to contain 4% β-hydroxy-γ-methyl glutamic acid and 3% albizzine, respectively. In the same study these and other nonprotein amino acids found in legume seeds were shown to be insecticidal or to repel the southern armyworm, *Prodenia eridania.*

A range of nonprotein amino acids from legume seed were later tested against *Callosobruchus maculatus* (Janzen et al., 1977). Mimosine, β-cyanoalanine, azetidine-2-carboxylic acid in the diet were lethal at 0.1% and canavanine and albizzine were lethal at 0.5%. Most nonprotein amino acids were more toxic than protein amino acids, although these too could be detrimental at high concen-



(1) (2) (3)

FIGURE 3. The structure of three aromatic nonprotein amino acids: (1) L-phenylalanine; (2) p-aminophenylalanine; (3) 3,4-dihydroxy-L-phenylalanine (L-DOPA).

trations. For example, when incorporated at 5% in the diet of *C. maculatus*, only 32% of protein amino acids were lethal compared with 90% of nonprotein amino acids.

The toxicity of nonprotein amino acids to bruchids is likely to be due at least in part to their ability to compete with protein amino acids in protein synthesis or other biosynthetic pathways involving protein amino acids. Experiments using radioactively labeled canavanine fed to a nonadapted insect, *Manduca sexta*, demonstrated that its protein synthesizing system cannot distinguish between arginine and its structural analogue canavanine, and that at least some of the canavanine, which is chemically less basic than arginine, is incorporated into protein in error. The biological activity of the protein is altered as a result, and the cell is unable to function normally. Tests on locusts have shown that canavanine is incorporated into the major protein of the fat body, which is important in egg development (Rosenthal, 1986). Nonprotein amino acids have been shown to have detrimental effects on insect growth, metamorphosis, fertility, and survival through feeding inhibition, neurotoxicity, enzyme inhibition, and interference with gut permeability and normal water balance (e.g., Harry et al., 1970; Schlesinger et al., 1976; Felton & Dahlman, 1984).

We have been studying the involvement of p-aminophenylalanine (a structural analogue of the protein amino acid phenylalanine) in the chemical defenses of wild *Vigna* seeds. This nonprotein amino acid is interesting because of its highly restricted taxonomic distribution. It has only been detected in seeds of five species from the genus *Vigna*, mostly from the small subgenus *Plectotropis* (Birch et al., 1985b). Although it is only present at levels between 0.1% and 1.3%, toxicity tests against two pest bruchids, *Callosobruchus maculatus* and *Zabrotes subfasciatus* showed that larval development was completely inhibited at concentrations of 0.75% and 0.3%, respectively. The difference in sensitivity to p-aminophenylalanine between *Z. subfasciatus* and *C. maculatus* suggests that the latter has partially overcome the seed defenses of *Vigna* species, to which it has been exposed over a longer evolutionary period than has *Z. subfasciatus*. The compound p-aminophenylalanine is also structurally related to another nonprotein amino acid, L-DOPA (see Fig. 3), which accumulates in seeds of *Mucuna* species at concentrations up to 10% (Bell & Janzen, 1971). Comparison of our data with Janzen's (1977a, b) indicates that p-aminophenylalanine is four and seven times as toxic to *C. maculatus* as L-DOPA and phenylalanine respectively.

Alkaloids. Seeds of many legume genera accumulate alkaloids, a heterogeneous range of basic nitrogenous compounds of low molecular weight that usually incorporate nitrogen in a heterocyclic

ring. More than 450 alkaloid structures have been identified from legume seeds (Kinghorn & Smolenski, 1981; Smolenski & Kinghorn, 1981). Genera containing alkaloid-accumulating species include *Acacia, Prosopis, Cassia, Sophora, Ormosia, Lupinus, Genista, Cytisus, Lotus, Mucuna, Abrus, Crotalaria,* and *Erythrina.*

Many alkaloids have marked dose-dependent pathologocial effects on insects and on their endosymbionts, manifested by retardation of growth, development, and reproduction as well as causing paralysis and mortality (Levinson, 1976). Some alkaloids, for example those with structures related to nicotine, may interfere with the nervous system by blocking receptor sites for acetylcholine (Corbett, 1974). Other possible mechanisms include interference with DNA replication, RNA transcription, protein synthesis, membrane transport processes, and enzyme functions (Robinson, 1979).

ENZYME INHIBITORS

Protease inhibitors. Many legume seeds have been shown to contain protease inhibitors (Janzen et al., 1986), some of which have been isolated and characterized in detail (Liener, 1973; Weder et al., 1983). These compounds can be harmful to humans and livestock, and plant breeding programs often select for their reduction or complete removal. All plant protease inhibitors studied so far are relatively small proteins with molecular weights ranging from about 4,000 to 8,000 daltons (Ryan, 1979). Although having amino acid sequences with a high degree of homology, they display considerable variation in active site specificities towards enzymes, including trypsin, chymotrypsin, and other proteases. Many insects are known to possess trypsinlike proteolytic enzymes in their guts. Initial studies suggested that the bruchids *Callosobruchus maculatus* and *C. chinensis* possessed only very low levels of protease activity, which were considered to be generally insensitive to legume seed inhibitors (Applebaum, 1964). However these assays were carried out at neutral or alkaline pH conditions primarily designed to detect serine-containing endopeptidases with activity similar to mammalian trypsin and chymotrypsin. Subsequent investigations have shown that *C. maculatus* larvae do possess active proteases, but they have an acidic pH optimum and are probably of a type more analogous to plant proteases, which depend on thiol groups for their activity (Gatehouse et al., 1985a, b).

Some legume seed protease inhibitors differ in their effect against different species of bruchid larvae. For example, *Vigna unguiculata* trypsin inhibitor isolated from IITA cultivar 2027 has some antimetabolic activity against *Callosobruchus maculatus* while trypsin inhibitors from wild *Vigna* species, *Lathyrus sativus,* chickpea, soybean, and lima bean are ineffective against this and other bruchid species (Birch et al., 1985b; Applebaum, 1964; Roy & Bhat, 1975; Janzen et al., 1977; Gatehouse & Boulter, 1983). The activity against bruchids of the protease inhibitors in cowpea 2027 may be due to their sequestering limiting sulphur amino acids in a form that is indigestible to larvae of *C. maculatus* (Gatehouse & Boulter, 1983). An additional possibility is that this cowpea seed also possesses other types of inhibitors, which may have been evolved by certain legumes as a defense against bruchids that utilize proteases insensitive to typical trypsin/chymotrypsin inhibitors found in many legume seeds (Gatehouse et al., 1985a, b).

Carbohydrase inhibitors. Inhibitors of mammalian amylase occur in many legume seeds and have been shown to be active against several stored-product pests, including flour beetles *(Tribolium confusum, T. castaneum),* yellow corn worm *(Tenebrio molitor),* and Mediterranean flour moth larvae *(Anagasta kuhniella)* (Powers & Culbertson, 1983). In preliminary tests against two pest bruchid species, we have found that commercially obtained purified *Phaseolus vulgaris* α-amylase inhibitor does have some detrimental effect on larvae at concentrations occurring naturally in seeds. Numbers of emerging adults of *Callosobruchus maculatus* and *Zabrotes subfasciatus* were reduced by 30% and 10%, respectively, compared with those on control diets, while mean duration of

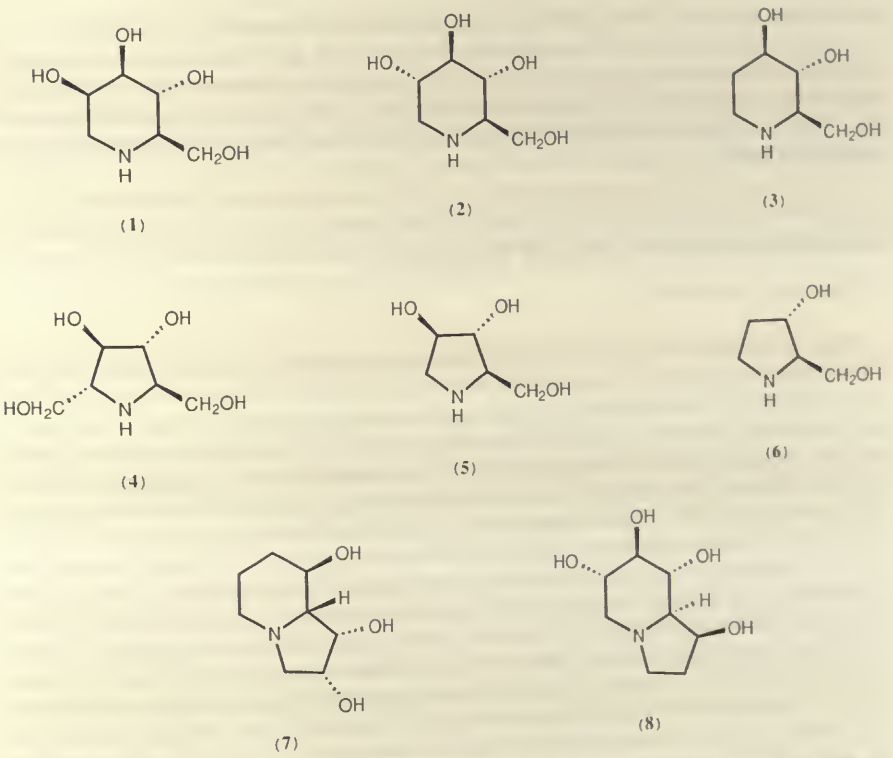


FIGURE 4. A range of polyhydroxyalkaloids known to be toxic to bruchids. (1) Deoxymannojirimycin. (2) Deoxynojirimycin. (3) Fagomine. (4) 2,5-dihydroxymethyl 3,4-dihydroxypyrrolidine (DMDP). (5) 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1). (6) 2-hydroxymethyl-3-hydroxypyrrolidine (CYB-3). (7) Swainsonine. (8) Castanospermine.

development was increased by 30% and 12% (Birch, unpublished). These preliminary findings suggest that these inhibitors of mammalian α-amylase, present in *P. vulgaris*, have some deleterious effects against nonadapted larvae, such as those of *C. maculatus*, which does not normally feed on kidney bean seeds. In contrast, the larvae of *Z. subfasciatus*, which readily attack kidney bean seeds, appear to have evolved some immunity to these inhibitors.

Biochemists and entomologists at our laboratory have recently been investigating another group of novel carbohydrase inhibitors, in particular glycosidase inhibitors, in legume seeds that have potent anti-bruchid activity. These compounds are polyhydroxy alkaloids and are interesting because several are structural analogues of sugar molecules. One, 2,5-dihydroxymethyl 3,4-dihydroxypyrrolidine (DMDP), an analogue of β-D-fructose (see Fig. 4), has been detected in seeds of several *Lonchocarpus* species at levels up to 6%. It is lethal to *Callosobruchus maculatus* larvae at 0.1% in the diet and slows larval development even at 0.01% (Evans et al., 1985). This study also found that larval α-D-glucosidase was strongly inhibited by DMDP in a competitive manner, although it had no significant effect on galactosidase or amylase. Several other polyhydroxy alkaloids with activity against bruchid glycosidase enzymes have been isolated from legume seeds. Castanospermine (see Fig. 4) occurs in seeds of *Castanospermum australe*, a native tree of subtropical Queensland and New South Wales, at a concentration of about 0.1%. This compound totally inhibits development of *C. maculatus* larvae when incorporated into the diet at only 0.03%,

and at levels as low as 0.005% significantly slows larval development (Nash et al., 1986). When it was tested against larval gut carbohydrases, α-D and β-D glucosidases were both strongly inhibited in a noncompetitive and competitive manner respectively (Gatehouse et al., 1985a). Other polyhydroxy alkaloids have been isolated from seeds of *C. australe* and *Angylocalyx* species (see Fig. 4), suggesting that this group of chemicals may be more widespread in legume seeds than originally thought.

Bruchid Adaptations to Pod and Testa Defenses

Behavioral Avoidance

Bruchids are likely to have evolved temporal and spatial mechanisms to avoid defenses associated with pods and testas. The specific oviposition tactics (Johnson, 1981) evolved by bruchids are likely to be closely synchronized with physical and chemical changes that occur during pod ripening and seed maturation. Behavioral adaptations that control the timing of oviposition could reduce larval mortality during penetration. This might be achieved by temporal avoidance of: high concentrations of pod or testa allelochemicals; fruit development stages when pod wall and testa barriers are of maximum hardness; and developing seeds when their nutritional value is suboptimal for development. Experiments on interactions between *Acanthoscelides obtectus* and its host *Phaseolus vulgaris* seeds have shown that oviposition behavior is apparently influenced more by the water content of the testa than by seed age. More eggs are laid on testas with high water content (20–40%), where frequency of larval penetration is relatively high, than on testas with low water content (6–10%), where larval penetration is reduced (Thiery, 1982). These results suggest that the ovipositing female can detect physical and chemical changes in the seed testa that correlate with the ability of her progeny to penetrate the seed. The time of larval penetration can also be altered by shortening or prolonging the normal embryo development within the egg. *Carydedon albonatatum* larvae delay hatching until seed maturation is complete (Prevett, 1966), while other bruchids have evolved very rapid embryonic development rates that enable larvae to enter pods before surface changes, such as flaking, occur (Center & Johnson, 1974).

Bruchids have also evolved tactics to overcome pod and testa defenses (Center & Johnson, 1974). For example, *Acanthoscelides fraterculus* lays its eggs into cracks beneath the flaking exocarp surfaces on pods of *Lotus crassifolius,* and *A. obtectus* chews holes into pod walls to oviposit inside the pods of *Phaseolus vulgaris.* The mouthparts of adult bruchids that chew holes into pod walls for oviposition may be specially modified, as are the mouthparts of specialist bruchid larvae like *Mimoesestes sallaei,* which can penetrate the tough testas of seeds that are impenetrable to generalists like *Callosobruchus maculatus* (Janzen, 1977b). Another form of spatial avoidance occurs in situations where penetration holes made by the first few successful larvae are then used by subsequent larvae (Labeyrie, 1960). The collective use of an existing hole may save an important amount of energy that may be critical in later stages of larval development, especially if further stress factors are imposed by defenses within the seed.

Perhaps the most important trait leading to behavioral avoidance of pod and testa allelochemicals is that of noningestion during larval penetration. This behavior has been proposed for *Callosobruchus maculatus* (Janzen, 1977b), *C. chinensis* (Southgate, 1984), *Sennius fallax* (Slansky, unpublished), and *Acanthoscelides obtectus* (Thiery, 1984) but may be a common trait in specialist and generalist bruchids. Detailed studies using the scanning electron microscope to investigate the contents of hatched *C. chinensis* eggs confirmed that they contain intact chiseled pieces of testa without any evidence of ingestion or mixing with saliva (Southgate, 1984). It has been suggested that this behavior could be induced by unidentified chemicals on the testa surface (Thiery, 1984) and perhaps also on the surface of pods. It has also been proposed that neonate larvae of some bruchids, including *A. obtectus,* may be better adapted than later instars, by being more responsive to these

surface chemical cues (Stamopoulos & Huiguard, 1980) although we have no evidence of this. Chemical investigations of these seed surfaces should reveal the types of compounds involved and whether the difference between larval noningestion and anti-penetration (i.e., biting deterrent) factors are qualitative or quantitative. Responses to these chemicals are also likely to differ among bruchid species. For example, the seed surface factors that inhibit testa penetration of *Phaseolus acutifolius* var. *latifolius* by *C. maculatus* larvae do not affect larvae of *Zabrotes subfasciatus* (Birch, unpublished). The latter bruchid is likely to have overcome this defense over a long period of association with the plant, since both beetle and plant are of New World origin. In contrast, *C. maculatus* is of Old World origin and has probably had little or no contact with these seeds during its evolutionary development (Birch et al., 1985b).

DETOXICATION AND OTHER COUNTERADAPTATIONS

Because so few chemical interactions between bruchids and legumes have been studied in any detail, very little is known about how these beetles deal with many of the potentially toxic chemicals found in legume seeds (see the section above on Specific Internal Defense Chemicals). The pioneering research of Rosenthal & Janzen (1983, 1985) investigated how the larvae of the bruchid *Caryedes brasiliensis* feed on seeds of *Dioclea megacarpa*, which contain up to 15% of the nonprotein amino acid canavanine, toxic for *Callosobruchus maculatus* at 1% (Janzen et al., 1977). This bruchid has been able to develop distinct but interrelated biochemical adaptations to utilize a potentially highly posionous phytochemical as a source of dietary nitrogen. These adaptations include: development of an enzyme capable of discriminating between arginine and its analogue canavanine during protein synthesis; formation of urea from canavanine coupled with formation of ammonia from urea; detoxication of the by-product canaline by enzymic conversion to homoserine; and increased affinity of larval arginase for canavanine compared with arginine. It thus seems probable that over a period of evolutionary time this bruchid has progressed from excretion of canavanine and canaline to making use of the compounds as a source of nitrogen. At least part of the ability of this bruchid to overcome and utilize canavanine may be due to microbial symbionts in the larval gut (Rosenthal, 1983).

Some specialist bruchids appear to have evolved biochemical mechanisms to deal with seed carbohydrase inhibitors. *Ctenocolum tuberculatum* feeds on seeds of *Lonchocarpus costaricensis* containing 2–6% DMDP. Preliminary tests on the α-glucosidase enzymes of adult *C. tuberculatum* indicated no inhibition by DMDP. In vitro tests on larval enzymes, however, indicated 50% inhibition of larval α-glucosidase, with 10^{-4} M DMDP in the specialist *C. tuberculatum*, and at 10^{-7} M in the generalist *Callosobruchus maculatus* (Fellows et al., unpublished).

A number of bruchid species are able to avoid the harmful effects of allelochemics by detoxifying them, as described in earlier sections, or by sequestering them. Larvae of the specialist bruchid *Bruchus atomarius* detoxify cyanogenic compounds in *Vicia sativa* seeds, yet larvae have very low levels of rhodanase, an enzyme usually associated with detoxification of those chemicals (Beesley et al., 1985). This bruchid clearly has evolved an alternative detoxification strategy, possibly using the enzyme β-cyanoalanine synthetase rather than rhodanase to metabolize cyanogenic compounds. Insects can detoxify alkaloids in various ways, including excretion in an unaltered form, metabolism to less toxic chemicals, sequestration, and possibly using protective barriers around the target nervous system (Dowd et al., 1983).

Several adaptive traits have been evolved by insects to overcome the anti-digestive properties of tannins. They include maintenance of a high gut pH (Bernays, 1981) and presence of detergent surfactants in the gut (Martin et al., 1985). The former trait is found in many Coleoptera and Lepidoptera, and the latter is now considered to be a widespread characteristic of many insect guts and may even be ubiquitous (Martin & Martin, 1984). The gut pH of *Callosobruchus maculatus* is apparently in the range of 4–6, which is the optimal range for many carbohydrase enzymes (Gate-

house et al., 1985a). It is therefore unlikely that this species is adapted to cope with tannins in this way. The presence of surfactants, like lysolecithin and linoleoglycine, has not been investigated in bruchids to our knowledge, but they have been found in at least one species of predatory beetle (Vonk, 1969). Other adaptive traits in insects that can counter the anti-digestive properties of tannins include possession of digestive β-glucosidase enzymes able to degrade hydrolyzable tannins, and peritrophic membranes that can absorb tannins and remove them from the gut (Bernays, 1981). However, because legume tannins present in pod walls and testas are likely to be chemically heterogeneous, one cannot generalize about their ecological role as defensive chemicals (Asquith & Butler, 1986). Even if bruchids have evolved mechanisms to counter their anti-digestive properties some tannins could alternatively be effective as larval mining inhibitors or could be directly toxic by their histopathological effects on the insect's gut wall, in a similar way to seed lectins (Gatehouse et al., 1984).

Ways of Maximizing Legume Chemical Defenses Against Bruchids

Genetic heterogeneity in plants and herbivores at inter- and intrapopulation levels has been well documented in recent reviews (e.g., Mitchell, 1983; Futuyama & Peterson, 1985), although the ecological significance of this variability is not always well understood. The capability of plant populations to counter herbivore adaptations depends in part on the former's genetic mechanisms for generating variation in defensive chemistry. Also, as a plant develops there will most likely be quantitative changes in the distribution and composition of defensive chemicals in different parts of the plant, including pod surface, pod wall, testa surface, testa interior, and cotyledon or endosperm.

QUANTITATIVE CHEMICAL VARIATION

The concentration of many secondary compounds accumulated within legume seeds varies between species and between and sometimes within populations of a single species. Quantitative variation often occurs between wild and domesticated forms of a single legume species, as in lima bean seeds where levels of HCN were found to be much higher in wild seeds than in cultivars (Janzen, 1977b). Even within domesticated selections, quantitative variation may exist. Significant seed-to-seed variation in the response of *Callosobruchus maculatus* to a resistant cultivated cowpea has been partially attributed to differing levels of protease inhibitor in different seed collections of the same variety (Dick & Credland, 1986).

Few studies have monitored quantitative changes in potential defensive chemicals during pod and seed development and ripening. Such a temporal chemical variability could have its greatest impact against bruchid attack if production of seeds were asynchronous for individual plants within a population (Janzen, 1969). One example of temporal variation in allelochemical content of legume seeds is provided by a comparison of trypsin inhibitor content in immature and mature winged beans *(Psophocarpus tetragonolobus;* Tadera et al., 1984). In four out of five accessions investigated, a large increase in inhibitor units per gram of seed (up to 100-fold) and per gram of protein (up to 7-fold) were found during seed ripening.

Spatial allocation of defensive compounds within seeds offers another source of quantitative variation to which bruchid larvae can be exposed. Localized concentrations of allelochemicals in the peripheral zone of the seed can effectively maximize defense potential for little extra metabolic cost. Most studies of potential anti-bruchid seed chemicals relate effectiveness in toxicity bioassays to total seed concentration of allelochemics and do not take account of the distribution within the legume seed. However, a few studies have investigated the distribution of allelochemicals within the seed. By scraping discrete layers from the outer and inner cotyledon surfaces, concentration zones of trypsin inhibitor activity have been detected in seeds of several legumes. Highest inhib-

itor activity was located on the outer cotyledon region of kidney bean, chickpea and soybean, while the reverse was found in peanut (Zimmerman et al., 1967; Barker et al., 1976). Increased trypsin inhibitor concentration in the outer cotyledon region has also been reported in a bruchid-resistant cowpea cultivar (Gatehouse & Boulter, 1983). There are also reports that lectins may be nonrandomly distributed in some legume seeds. In *Phaseolus vulgaris* and *Ricinus communis* seeds, for example, lectins have been found to accumulate in small protein-filled vacuoles called protein bodies in large parenchyma cells of the cotyledons (Barker et al., 1976; Tully & Beevers, 1976).

We have noted the nonrandom distribution of fixed oil globules around the outer cotyledon surfaces of *Lonchocarpus* species. Although the chemical content and ecological function of the oils remain to be elucidated, they may represent an example of a localized chemical barrier against seed-eating insects, since many seed oils are known to be toxic to a range of organisms (Seigler, 1979). The relative proportions of biosynthetically related allelochemicals can vary within a plant population. Such qualitative variation has been observed with alkaloids in lupines, where it has been found that the most effective chemical defence against insect attack was observed in a population that contained a small number of different alkaloids in relatively high concentration and that had high individual variability (Dolinger et al., 1973).

This chapter has considered single classes of defense chemicals, but obviously any insect predator encounters a complex mix of chemicals as many plants will produce secondary compounds from more than one chemical class. Also, there is a strong selective advantage for plants to produce several lines of chemical defenses, especially if they can interact synergistically. Such combinations of chemicals are more difficult for the insect to circumvent (Berenbaum, 1985).

Summary

This review is based on recent literature on bruchid–legume interactions, which is an expanding area of research. It describes some of the principles associated with host finding by bruchids and the suitability of legume hosts for bruchid development. The success or failure of a bruchid to establish itself on a legume can be the consequence of chemical and physical factors operating at any of five stages in its life cycle: habitat selection, host finding, host recognition, host acceptance, and host suitability. Factors influencing all of these stages are examined in detail, including aspects of plant biology that militate against penetration of the pod and testa by bruchid larvae. Behavioral and metabolic adaptations of bruchids that enable them to circumvent the defenses of pods and testas are considered.

There is a need for further experimental work and field studies in all aspects of bruchid–legume predation. Because of the importance of legumes as crops in developing and developed countries alike future research should look for ways of modifying and improving legume agriculture. These investigations should include behavioral and developmental studies designed to identify vulnerable or exploitable aspects of the bruchid–legume interaction. Emphasis should be placed on the search for novel allelochemics and for multifactor protection: both are less likely to be overcome rapidly. It will be particularly important to study wild varieties of crop legumes in the search for protective traits compatible with crop status that may be incorporated into crop varieties by selective breeding or genetic engineering.

References

Akingbohungbe, A. E. 1976. A note on the relative susceptibility of unshelled cowpeas to the cowpea weevil *(Callosobruchus maculatus)*. Trop. Grain Legume Bull. 5: 11–13.

Alzouma, I. 1981. Observations on the ecology of *Bruchidius atrolineatus* and *Callosobruchus maculatus* in Niger. Pp. 205–213 *in* V. Labeyrie (editor), The Ecology of Bruchids Attacking Legumes. Junk, The Hague.

Applebaum, S. W. 1964. Physiology aspects of host specificity in the Bruchidae. 1. General considerations of development compatibility. J. Insect Physiol. 10: 783–788.

_____ & Y. Birk. 1972. Natural mechanisms of resistance to insects in legume seeds. Pp. 629–636 *in* J. G. Rodriguez (editor), Insect and Mite Nutrition. North-Holland Publishers, Amsterdam.

_____ & _____. 1979. Saponins. Pp. 539–562 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interactions with Secondary Plant Metabolites. Academic Press, New York.

_____ & M. Guez. 1972. Comparative resistance of *Phaseolus vulgaris* beans to *Callosobruchus chinensis* and *Acanthoscelides obtectus* (Col. Bruchidae): the differential digestion of soluble heteropolysaccharide. Entomol. Exp. Appl. 25: 64–74.

_____, B. Gestetner & Y. Birk. 1965. Physiological aspects of host specificity in the Bruchidae IV. Developmental incompatability of soybeans for *Callosobruchus*. J. Insect Physiol. 11: 611–616.

_____, S. Marco & Y. Birk. 1969. Saponins as possible factors of resistance of legume seeds to the attack of insects. Agric. & Food Chem. 17(3): 18–622.

_____, U. Tadmor & H. Podoler. 1970. The effect of starch and of a heteropolysaccharide fraction from *Phaseolus vulgaris* on development and fecundity of *Callosobruchus maculatus*. Entomol. Exp. Appl. 13: 61–70.

Asquith, T. N. & L. B. Butler. 1986. Interactions of condensed tannins with selected proteins. Phytochemistry 25: 1591–1593.

Barker, R. J., E. Derbyshire, A. Yarwood & D. Boulter. 1976. Purification and characterisation of the major storage proteins of *Phaseolus vulgaris* seeds, and their intracellular and cotyledonary distribution. Phytochemistry 15: 751–757.

Beesley, S. G., S. G. Compton & D. A. Jones. 1985. Rhodanese in insects. J. Chem. Ecol. 11(1): 45–49.

Bell, E. A. & D. H. Janzen. 1971. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. Nature 229: 136–137.

Berenbaum, M. 1985. Brementown revisited: interactions among allelochemicals in plants. Pp. 139–169 *in* G. A. Cooper-Driver, T. Swain & E. E. Conn (editors), Chemically Mediated Interactions between Plants and Other Organisms. Plenum Press, New York.

Bernays, E. A. 1978. Tannins: an alternative viewpoint. Entomol. Exp. Appl. 24: 44–53.

_____. 1981. Plant tannins and insects herbivores: an appraisal. Ecol. Entomol. 6: 353–360.

Biermont, J. C. & A. Bonet. 1981. The bean weevil populations from the *Acanthoscelides obtectus* Say. group living on wild or subspontaneous *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. and on *Phaseolus vulgaris L.* cultivated in the Tepoztlan region state of Morfelos-Mexico. Pp. 23–41 *in* V. Labeyrie (editor), The Ecology of Bruchids Attacking Legumes (Pulses). Junk, The Hague.

_____, G. Chauvin & J. F. Germain. 1982. L'oeuf de *Bruchidius atrolineatus* (Pic) et son système de fixation. Canad. J. Zool. 60: 2610–2615.

Birch, N., L. Crombie & W. M. Crombie. 1985a. Rotenoids of *Lonchocarpus salvadorensis:* their effectiveness in protecting seeds against bruchid predation. Phytochemistry 24(12): 2881–2883.

_____, B. J. Southgate & L. E. Fellows. 1985b. Wild and semi-cultivated legumes as potential sources of resistance to bruchid beetles for crop breeders: study of *Vigna/Phaseolus*. Pp. 303–320 *in* G. E. Wickens, J. R. Goodin & D. V. Field (editors), Plants for Arid Lands. Royal Botanic Gardens, Kew.

_____, L. E. Fellows, S. V. Evans & S. V. Docherty. 1986. Para-aminophenylalanine in *Vigna:* possible taxonomic and ecological significance as a seed defence against bruchids. Phytochemistry 25: 2745–2751.

Blaney, W. M. 1980. Chemoreception and food selection by locusts. Pp. 127–130 *in* H. Van der Starre (editor), Olfaction and Taste VII. IRL Press, London.

_____, C. Winstanley & M. S. J. Simmonds. 1985. Food selection by locusts: an analysis of rejection, behaviour. Entomol. Exp. Appl. 38: 35–40.

_____, M. S. J. Simmonds, S. V. Evans & L. E. Fellows. 1984. The role of the secondary plant compound 2,5-dihydroxymethyl 3,4-dihydroxypyrrolidine as a feeding inhibitor for insects. Entomol. Exp. Appl. 36: 209–216.

_____, _____, S. V. Ley & R. B. Katz. 1987. An electrophysiological and behavioural study of insect antifeedant properties of natural and synthetic drimane-related compounds. Physiol. Entomol. 12: 281–291.

Booker, R. H. 1967. Observations on three bruchids associated with cowpea in northern Nigeria. J. Stored Prod. Res. 3: 1–15.

Boughdad, A., Y. Gillon & C. Gagnepain. 1986. Influence des tannins condensés du tégument de fèves *(Vicia faba)* sur le développment larvaire de *Callosobruchus maculatus*. Entomol. Exp. Appl. 42: 125–132.

Brett, C. H. 1946. Insecticidal properties of the indigo bug *(Amorpha fructicosa)*. J. Agric. Res. 73: 81–96.
Bridwell, J. C. 1918. Notes on the Bruchidae and their parasites in the Hawaiian Islands. Proc. Hawaiian Entomol. Soc. 3: 4365–4505.
_____. 1920. Notes on the Bruchidae and their parasites in the Hawaiian Islands. Proc. Hawaiian Entomol. Soc. 4: 403–409.
Caswell, G. H. 1960. Observation on an abnormal form of *Callosobruchus maculatus* (F.) Bull. Entomol. Res. 50: 671–680.
_____. 1984. The value of the pod in protecting cowpea seed from attack by bruchid beetles. Samara J. Agric. Res. 2: 49–55.
Center, T. D. & C. D. Johnson. 1974. Coevolution of some beetles (Coleoptera: Bruchidae) and their hosts. Ecology 55: 1096–1103.
Corbett, J. R. 1974. The Biochemical Mode of Action of Pesticides. Academic Press, New York.
Cushman, R. A. 1911. Notes on the host plants and parasites of some North American bruchidae. J. Econ. Entomol. 4: 489–510.
De Luca, Y. 1973. Comportement d'inactivité imaginale de Bruchides. (Col.). Ann. Soc. Mort. Hist. Nat. 113: 79–83.
Dick, K. M. & P. F. Credland. 1986. Variation in the response of *Callosobruchus maculatus* to a resistant variety of cowpea. J. Stored Prod. Res. 22(1): 43–48.
Dixon, A. F. D. 1985. Aphid Ecology. Blackie, London.
Dobie, P., J. V. Greve, K. Hoth & A. M. Kilminster. 1979. Inability of storage Bruchidae to infest winged beans *(Psophocarpus tetragonolobus)*. Entomol. Exp. Appl. 26: 168–174.
Dolinger, P. M., P. R. Ehrlich, W. L. Fitch & D. E. Breedlove. 1973. Alkaloid and predation patterns in Colorado lupine populations. Oecologia 13: 191–204.
Dowd, P. F., C. M. Smith & T. C. Sparks. 1983. Detoxification of plant toxins by insects. Insect Biochem. 13: 453–468.
Evans, S. V., A. M. R. Gatehouse & L. E. Fellows. 1985. Detrimental effects of 2,5-dihydroxymethyl-3, 4-dihydroxypyrrolidine in some tropical legume seeds on larvae of the bruchid *Callososbruchus maculatus*. Entomol. Exp. Appl. 37: 257–261.
Fatunla, T. & K. Badura. 1983. Resistance of cowpea pods to *Callosobruchus maculatus*. J. Agric. Sci. 100: 205–209.
Feeny, P. P. 1975. Biochemical coevolution between plants and their insect herbivores. Pp. 3–19 *in* L. E. Gilbert & P. H. Raven (editors), Coevolution of Animals and Plants. Univ. of Texas Press, Austin, Texas.
_____. 1976. Plant apparency and chemical defence. Recent Adv. Phytochem. 10: 1–40.
Felton, G. W. & D. C. Dahlman. 1984. Allelochemical induced stress: effects of L-canavanine on the pathogenicity of *Bacillus thuringiensis* in *Manduca sexta*. J. Invert. Pathol. 44: 187–191.
Fenwick, D. E. & D. Oakenfall. 1983. Saponin content of food plants and some prepared foods. J. Sci. Food Agric. 34: 186–191.
Fukami, H. & M. Nakajima. 1971. Rotenone and the rotenoids. *In:* M. Jacobson & D. G. Crosby (editors), Naturally Occurring Insecticides.
_____, T. Nakatsugawa & T. Narahashi. 1959. The relation between chemical structure and toxicity in rotenone structures. Japanese J. Appl. Entomol. Zool. 3: 259–265.
Futuyma, D. J. & S. C. Peterson. 1985. Genetic variation in the use of resources by insects. Ann. Rev. Entomol. 30: 217–238.
Gatehouse, A. M. R. & D. Boulter. 1983. Assessment of the antimetabolic effects of trypsin inhibitors from cowpea *(Vigna unguiculata)* and other legumes on development of the bruchid beetle *Callosobruchus maculatus*. J. Sci. Food Agric. 34: 345–350.
_____, K. A. Fenton & J. H. Anstee. 1985a. Carbohydrase and esterase activity in the gut of larval *Callosobruchus maculatus*. Experientia 41: 1201–1203.
_____, K. J. Butler, K. A. Fenton & J. A. Gatehouse. 1985b. Presence and partial characterisation of a major proteolytic enzyme in the larval gut of *Callosobruchus maculatus*. Entomol. Exp. Appl. 39: 279–286.
_____, F. M. Dewey, J. Dove, K. A. Fenton & A. Pusztai. 1984. Effect of seed lectins from *Phaseolus vulgaris* on the development of larvae of *Callosobruchus maculatus*; mechanism of toxicity. J. Sci. Food Agric. 35: 373–380.
Germain, J. F., J. Huigard & J. P. Monge. 1985. Influence des inflorescences de la plante hôte *(Vigna unguiculata)* sur la levée de la diapause reproductice de *Bruchidius atrolineatus*. Entomol. Exp. Appl. 39: 35–42.

GRIFFITHS, D. W. 1981. The polyphenolic content and enzyme inhibitory activity of testas from bean *(Vicia faba)* and pea *(Pisum* spp.) varieties. J. Sci. Food Agric. 32: 797–804.

GUNN, C. R. 1981. Seeds of Leguminosae. Pp. 913–925 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

HARLAN, J. 1976. Genetic resources in wild relatives of crops. Crop Sci. 16: 329–333.

HARRY, P., Y. DROR & S. W. APPLEBAUM. 1970. Arginase activity in *Tribolium casteneum* and the effect of canavanine. Insect Biochem. 6: 273–279.

HINCKLEY, A. D. 1960. The klu beetle, *Mimosestes sallaei* (Sharp), in Hawaii (Coleoptera: Bruchidae). Proc. Hawaiian Entomol. Soc. 17: 260–269.

HONDA, H., K. OSHIMA & I. YAMAMOTO. 1976. Oviposition marker of bean weevil. Pp. 116–128 *in* Proc. U.S. Japan Seminar Stored Prod. Pests.

INGHAM, J. L. 1983. Naturally occurring isoflavonoids (1855–1981). Pp. 98–109 *in* W. Herz, H. Grisebach & G. W. Kirby (editors), Progress in the Chemistry of Organic Natural Products, Volume 43. Springer-Verlag, New York.

ISHII, S. 1952. Studies on the Host Preference of Cowpea Weevil (*Callosobruchus chinensis* L.). Bull. Nat. Inst. Agric. Sci. Japan 1(C): 185–256.

JANZEN, D. H. 1969. Seedeaters vs. seed size, number, toxicity and dispersal. Evolution 23: 1–27.

———. 1976a. Reduction of *Mucuna andreana* (Leguminosae) seedling fitness by artificial seed damage. Ecology 57: 826–828.

———. 1976b. Insecticidal action of the phytohaemaglutinin in black beans on a bruchid beetle. Science 192: 795–796.

———. 1977a. The interaction of seed predators and seed chemistry. Coll. Int. C. N. R. S. 265: 414–418.

———. 1977b. How southern cowpea weevil larvae (Bruchidae: *Callosobruchus maculatus)* die on nonhost seeds. Ecology 58: 921–927.

———. 1980. Specificity of seed-attacking beetles in a Costa Rican deciduous forest. J. Ecology 68: 929–952.

———, H. B. JUSTER & E. A. BELL. 1977. Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle *Callosobruchus maculatus*. Phytochemistry 16: 223–227.

———, C. A. RYAN, I. E. LIENER & G. PEARCE. 1986. Potentially defensive proteins in mature seeds of 59 species of tropical Leguminosae. J. Chem. Ecol. 12: 1469–1481.

JARRY, M. 1981. Evolution of spatial pattern of attacks by *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) of *Phaseolus vulgaris* L. pods in South West France. Pp. 131–141 *in* V. Labeyrie (editor), The Ecology of Bruchids Attacking Legumes (Pulses). Junk, The Hague.

JOHNSON, C. D. 1970. Biosystematics of the Arizona, California and Oregon species of the seed beetle genus *Acanthoscelides* Schilsky (Coleoptera: Bruchidae). Univ. California Publ. Entomol. 59: 1–116.

———. 1981. Seed beetle host specificity and the systematics of the Leguminosae. Pp. 995–1027 *in* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew.

KEARNEY, T. H. & R. H. PEEBLES. 1960. Arizona Flora. Univ. California Press, Berkeley & Los Angeles.

KENNEDY, J. S. & J. E. MOORHOUSE. 1969. Laboratory observations on locust responses to wind-bourne grass odour. Entomol. Exp. Appl. 12: 487–503.

———, C. O. BOOTH & W. S. J. KERSHAW. 1961. Host finding by aphids in the field. iii. Visual attraction. Ann. Appl. Biol. 12: 487–503.

KINGHORN, A. D. & S. J. SMOLENSKI. 1981. Alkaloids of Papilionoideae. Pp. 585–598 *in* R. H. Polhill & P. H. Raven (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

LABEYRIE, V. 1960. Nature de la répartition des larves d'*Acanthoscelides obtectus* Say. C.R. Acad. Sci. 251: 149–151.

LEROI, B. & M. JARRY. 1981. Relation d'*Acanthoscelides obtectus* avec différentes especès de *Phaseolus:* influence sur la fécondité et possibilitiés de dévelopment larvaire. Entomol. Exp. Appl. 30: 173–182.

———, C. CHARARAS & J. M. CHIPOULET. 1984. Étude des activités ocidasiques du tube digestif des adults et des larves de la bruche du haricot, *Acanthoscelides obtectus*. Entomol. Exp. Appl. 35: 269–273.

LEVINSON, H. Z. 1976. The defensive role of alkaloids in insects and plants. Experientia 32: 408–410.

LIENER, I. E. 1973. Toxic factors associated with legume proteins. Ind. J. Nutr. Dietet. 10: 303–321.

MA, W. C. 1977. Alterations in chemoreceptor function in armyworm larvae *(Spodoptera exempta)* by a plant-derived sesquiterpenoid and by sulphydryl reagents. Physiol. Entomol. 2: 199–207.

MARTIN, M. M. & J. S. MARTIN. 1984. Surfactants: their role in preventing the precipitation of proteins by tannins in insect guts. Oecologia 61: 342–345.

_____, D. C. Rockholm & J. S. Martin. 1985. Effects of surfactants, pH and certain cations on precipitation of proteins by tannins. J. Chem. Ecol. 11: 485–494.

McKey, D. 1979. The distribution of secondary compounds within plants. Pp. 56–133 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Messina, F. J. 1984. Influence of cowpea maturity on the oviposition choices and larval survival of a bruchid beetle *Callosobruchus maculatus*. Entomol. Exp. Appl. 35: 241–248.

_____ & J. A. A. Renwick. 1985. Resistance to *Callosobruchus maculatus* (Coleoptera: Bruchidae) in selected cowpea lines. Environ. Entomol. 14: 868–872.

Miller, R. 1967. *Crotalaria* seed morphology, anatomy, and identification. U.S.D.A. Techn. Bull. 1373: 1–73.

Mitchell, R. 1983. Effects of host–plant variability on the fitness of sedentary herbivorous insects. Pp. 343–370 *in* R. F. Denno & M. S. MaClure (editors), Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, London.

Mulkern, G. B. 1969. Behavioural influences on food selection in grasshoppers (Orthoptera: Acrididae), Entomol. Exp. Appl. 12: 509–523.

Nash, R. J., K. A. Fenton, A. M. R. Gatehouse & E. A. Bell. 1986. Effects of the plant alkaloid castanospermine as an antimetabolite of storage pests. Entomol. Exp. Appl. 42: 71–77.

Nwanze, K. F. & E. Horber. 1976. Seed coats of cowpeas affect oviposition and larval development of *Callosobruchus maculatus*. Environ. Entomol. 5: 213–218.

Oshima, K., H. Honda & I. Yamamoto. 1973. Isolation of an oviposition marker from azuki bean weevils, *Callosobruchus chinensis* (L.). Agric. Biol. Chem. 37: 2679–2680.

Pajni, H. R. 1981. Trophic relations and ecological status of the adults of *Bruchus pisorum* L. and allied field species of Bruchidae (Coleoptera). Pp. 125–129 *in* V. Labeyrie (editor), The Ecology of Bruchids Attacking Legumes (Pulses). Junk, The Hague.

_____ & S. Sood. 1975. Effect of pea pollen feeding on maturation and copulation in the beetle *Bruchus pisorum*. Indian J. Exp. Biol. 13: 202–203.

Pimbert, M. P. & D. Pierre. 1983. Ecophysiological aspects of bruchid reproduction. 1. The influence of pod maturity and seeds of *Phaseolus vulgaris* and the influence of insemination on the reproductive activity of *Zabrotes subfasciatus*. Ecol. Entomol. 8: 87–94.

Podoler, H. & S. W. Applebaum. 1968. Physiological aspects of host specifity in the Bruchidae. V. Varietal differences in the resistance of *Vicia faba* L. to *Callosobruchus chinensis* L. J. Stored Prod. Res. 4: 9–11.

_____ & _____. 1971. Host specificity in the Bruchidae VII. The effect of carbohydrate composition on varietal resistance of garden peas to *Callosobruchus chinensis* L. J. Stored Prod. Res. 7: 97–105.

Pouzat, J. 1978. La régulation de l'activité reproductrice des femelles de Bruche du haricot *(Acanthoscelides obtectus* Say): influence de quelques facteurs extremes (plante hôte, activité locomotrice). Thèse d'Etat, Univ. F. Rabelais Tours.

Powers, J. R. & J. D. Culbertson. 1983. Interaction of a purified bean (*Phaseolus vulgaris*) glycoprotein with an insect amylase. Cereal Chem. 60: 427–429.

Prevett, P. F. 1961. Field infestations of cowpea (*Vigna unguiculata*) pods by beetles of the families Bruchidae and Curculionidae in northern Nigeria. Bull. Entomol. Res. 52: 635–645.

_____. 1966. Observations on biology in the genus *Caryedon* in northern nigeria with a list of associated parasitic Hymenoptera. Proc. R. Entomol. Soc. London 41: 9–16.

_____. 1967. Observation on the biology of six species of Bruchidae in northern Nigeria. Entomol. Monthly Mag. 102: 174–180.

Pusztai, A., E. M. W. Clarke & T. P. King. 1979. The nutritional toxicity of *Phaseolus vulgaris* lectins. Nutr. Soc. 38: 115–120.

Reed, W., S. S. Lateef & S. Sithanatham. 1980. Are bruchids field pests of chickpea? Int. Chickpea Newsletter 3: 16.

Rehr, S. S., E. A. Bell, D. H. Janzen & P. P. Feeny. 1973. Insecticidal amino acids in legume seeds. Biochem. Syst. 1: 63–67.

Rembold, H. 1981. Malic acid in chickpea exudates—marker for *Heliothis* resistance. Int. Chickpea Newsletter 4: 18–19.

Rhoades, D. F. 1979. Evolution of plant chemical defence against herbivores. Pp. 4–48 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Metabolites. Academic Press, New York.

_____ & R. G. Cates. 1976. Towards a general theory of plant antiherbivore chemistry. Recent Adv. Phytochem. 11: 168–213.

Robinson, T. 1979. The evolutionary ecology of alkaloids. Pp. 413–442 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Metabolites. Academic Press, New York.

Root, R. B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collard *(Brassica oleracea)*. Ecol. Monogr. 43: 95–120.

Rosenthal, G. A. 1983. A seed-eating beetle's adaptation to a poisonous seed. Sci. Amer. (Nov. 1983): 164–171.

_____. 1986. Biochemical insight into insecticidal properties of L-canavanine, a higher plant protective allelochemical. J. Chem. Ecol. 12: 1145–1156.

_____ & E. A. Bell. 1979. Naturally occurring, toxic nonprotein amino acids. Pp. 333–385 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

_____ & D. H. Janzen. 1983. Avoidance of nonprotein amino acid incorporation into protein by the seed predator *Caryedes brasiliensis*. J. Chem. Ecol. 9: 1353–1361.

_____ & _____. 1985. Ammonia utilization by the bruchid beetle, *Caryedes brasiliensis*. J. Chem. Ecol. 11: 539–544.

Roy, D. A. & R. V. Bhat. 1975. Variation in neurotoxin, trypsin inhibitors and susceptibility to insect attack in varieties of *Lathyrus sativus* seeds. Environ. Physiol. Biochem. 5: 172–177.

Ryan, C. A. 1979. Proteinase inhibitors. Pp. 599–617 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Schlesinger, H. M., S. W. Applebaum & Y. Birk. 1976. Comparative uptake, tissue permeability and turnover of alpha, gamma, diaminobutyric acid and beta-cyanoalanine in locusts. J. Insect Physiol. 23: 1311–1313.

Schoonhoven, A. V., C. Cardona & J. F. Valor. 1983. Resistance to the bean weevil and Mexican bean weevil in noncultivated common bean accessions. J. Econ. Entomol. 76: 1255–1259.

Seigler, D. S. 1979. Toxic seed lipids. Pp. 449–466 *in* G. A. Rosenthal & D. H. Janzen (editors), Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Serrano, M. S., A. V. Schoonhoven, J. F. Valor & C. Cardona. 1983. Fuentes da resistencia en materiales silvestres de frijol al ataque de gorgojo comun del frijol, *Acanthoscelides obtectus* (Say). Rev. Columbia Entomol. 9: 13–18.

Simmonds, M. S. J. & W. M. Blaney. 1984. Some effects of azadirachtin on lepidopterous larvae. Pp. 163–180 *in* H. Schmutterer & K. R. S. Ascher (editors), Proc. 2nd Int. Neem Conf. GTZ Eschborn.

Smolenski, S. J. & A. D. Kinghorn. 1981. Alkaloids of Caesalpinioideae and Mimosoideae. Pp. 579–584 *in* R. H. Polhill & P. H. Ravan (editors), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Southgate, B. J. 1984. Observations on the larval emergence in *Callosobruchus chinensis*. Entomol. Genet. 9: 177–180.

Stamopoulos, D. & J. Huignard. 1980. L'influence des diverses parties de la graine de haricot *(Phaseolus vulgaris)* sur le dévelopment des larves d'*Acanthoscelides obtectus*. Entomol. Exp. Appl. 28: 38–46.

Stanton, M. L. 1983. Spatial patterns in the plant community and their effects upon insect searching behaviour. Pp. 125–157 *in* S. Ahmed (editor), Herbivorous Insects Host Seeking Behavior and Mechanisms. Academic Press, London.

Sternly, B. A. & M. A. Berenbaum. 1985. Histopathological effects of tannins on the midgut epithelium of *Papilio polyxenes*. Entomol. Exp. Appl. 39: 3–9.

Sutherland, D. R. W., N. D. Hood & J. R. Hillier. 1975. Lucerne root saponins: a feeding deterrent for the grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). New Zealand J. Zool. 2: 93–100.

Swain, T. 1965. The tannins. Pp. 553–580 *in* J. Bonner & J. E. Varner (editors), Plant Biochemistry. Academic Press, New York.

_____. 1973. Biochemical evolution in plants. Pp. 125–302 *in* M. Flokin & E. H. Stotz (editors), Comprehensive Biochemistry, Volume 29a. Elsevier, Amsterdam.

Tadera, K., T. Taniguchi, M. Ariona, F. Yagi, A. Kobayashi & K. Ishihata. 1984. Protein and trypsin and trypsin inhibitor in immature pods of winged bean, *Psophocarpus tetragonolobus*. Mem. Fac. Agric. Kagoshia Univ. 20: 63–71.

Thiery, D. 1982. Influence de la teneur en eau et du dureté du tégument des graines de *Phasoleus vulgaris* sur la fréquence de pénétration des larvae néonates d'*Acanthoscelides obtectus*. Entomol. Exp. Appl. 32: 141–145.

_____. 1984. Hardness of some fabaceous seed coats in relation to larval penetration by *Acanthoscelides obtectus* (Say). J. Stored Prod. Res. 20: 77–181.

_____ & M. Jarry. 1985. Hatching rhythm in the bean weevil *Acanthoscelides obtectus* and larval penetration of *Phaseolus vulgaris* seeds. Insect Sci. Applic. 6: 33–35.

Tookey, H. L., V. F. Pfiefer & L. R. Martin. 1963. Gums separated from *Crotalaria intermedia* and other leguminous seeds by dry milling. Agric. Food Chem. 11: 317–321.

Tully, R. E. & H. Beevers. 1976. Protein bodies of castor bean endosperm. Isolation, fractionation, and the characterization of protein components. Pl. Physiol. 58: 710–715.

Utida, S. 1972. Density-dependent polymorphism in the adult of *Callosobruchus maculatus* (Coleoptera, Bruchidae). J. Stored Prod. Res. 8: 111–126.

Visser, J. H. 1986. Host odour perception in phytophagous insects. Ann. Rev. Entomol. 31: 121–144.

Vonk, H. J. 1969. The properties of some emulsifiers in the digestive fluids of invertebrates. Comp. Biochem. Physiol. 29: 361–371.

Wasserman, S. S. 1981. Host induced oviposition preferences and oviposition markers in the cowpea weevil, *Callosobruchus maculatus*. Ann. Entomol. Soc. Amer. 74: 242–245.

_____ & D. J. Futuyma. 1981. Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). Evolution 35: 605–617.

Weder, J. K. P., M. P. Hegarty, M. Holzner & M. L. Kirn-Dirndorfer. 1983. Trypsin and chymotrypsin inhibitors in Leguminosae. Zeitsch. Lebens. Forsch. 177: 109–113.

Yamamoto, I. 1970. Mode of action of pyrethroids, nicotinoids and rotenoids. Ann. Rev. Entomol. 15: 262–265.

Zachariae, G. van. 1958. Das Verhalten des Speisebohnenkaters *Ananthoscelides obtectus* Say in Freien in Norddeutschland. Z. Angew Entomol. 43: 345–365.

Zimmerman, G., S. Weissman & S. Yannai. 1967. The distribution of protein, lysine and methionine, and antitryptic activity in the cotyledons of some leguminous seeds. J. Food Sci. 32: 129–130.

DATABASES, INFORMATION SYSTEMS, AND LEGUME RESEARCH

Frank A. Bisby[1]

ABSTRACT

Databases and information systems can enlarge the range and efficiency of legume researchers' work by providing modern information support services. Some progress is now being made on access to factual data of many kinds (including agronomic, chemical, and ecological data), taxonomic indexing, identification assistance, access to the literature, access to analytical techniques for application to the factual data, access to germplasm, and rapid communications among legume workers. ILDIS (International Legume Database and Information Service) was established in September 1985 as an international organization to set up a legume database and a communications network for use by legume workers and others, including international agencies in conservation, administration, environment, and germplasm resources. The first two phases of the project involve a geographic/taxonomic checklist system for all legume species, and detailed factual modules for species in 16 of the 42 legume tribes selected for economic importance as food, forage, industrial gums, and timber. Services will include on-line access, distribution on disc, a postal enquiry service, and publications. Modern developments in artificial intelligence, communications, and international networks should provide yet more advances in the information provision and communications among legume researchers, provided adequate resources are devoted to system design and to software development.

I. INTRODUCTION

Databases and information systems may not themselves generate new discoveries in the biology of legumes: what they can do is enlarge the range and efficiency of legume researchers' work by providing modern information support services. In reviewing the development of biological databases and information systems relevant to legume research, we again come upon two themes that have driven this conference: the interface of systematics with biology and the need to think big.

The interface of systematics with biology can be thought of in a retail model (Bisby, 1984), where activities of the taxonomic profession consist of internal services intended for other taxonomists and external services that actually produce a product for use outside the profession. These external services may take many forms (floras, monographs, educational events), but the essential components are classification, nomenclature, descriptions, and aids to identification. Databases are being built to store this essential taxonomic reference system; they are being introduced in a new generation of sophisticated information systems, which are at the interface between systematics and the rest of biology. They are at the interface in two senses: 1) that the systematic information is generated by taxonomists for other biologists, and 2) that the more sophisticated systems demand collaboration in linking the reference system provided by taxonomists with information from other botanical sources. Examples of the latter would be information from floral biology or from phytochemistry.

We must think big: computer science and information technology are moving rapidly and we are on the verge of a revolution in the communication and presentation of botanical information. We need to think of international networks and electronic communications that will allow botanists in different countries to collaborate in collating and disseminating information. We need to experiment with imaginative novel styles and features of presentation to develop the services. Projects needed

[1]Biology Department, Building 44, University of Southampton, Southampton, SO9 5NH, England.

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to test these concepts should be small pilot schemes but in design they must envisage wide participation, wide usage, and large resources of data. The features that interest us will probably be: access to factual data of many kinds (including agronomic, chemical, and ecological data), taxonomic indexing, identification assistance, access to the literature, access to analytical techniques for application to the factual data, access to germplasm, and rapid communications among legume workers.

II. Development of Information Systems in Legume Biology

In discussing the development of information systems in legume biology, we should distinguish certain types of systems. Most databases are *structured* rather than textual, that is, the data are organized into structured elements where some of the meaning is incorporated in the structure and known to the software. Only in a few cases is it purely textual, as in the use of wordprocessing in preparation of a flora or monograph, or as just part of a database, as in the IUCN (International Union for the Conservation of Nature) Threatened Plants System where, in addition to a structured database, there are text documents for certain species and countries (Mackinder, 1984). We should distinguish *bibliographic, factual,* and *image* content: are the data bibliographic citations, botanical facts, or images? And among the factual systems, what are the units to which the facts relate? In the older and simpler factual systems we can clearly distinguish *specimen-based* systems, referring to herbarium specimens, specimen plants in botanic gardens, or germplasm collections; *name-based* systems giving nomenclatural data for names; and *taxon-based* systems giving botanical data about taxa (Bisby, 1984; Abbott et al., 1985).

Early flat-file systems

Early systems started in the late 1960s and in the 1970s mostly comprised a single flat-file (Brill & Estabrook, 1984) which can be thought of as a file of records, such as those often put on index cards, or as a large rectangular data table with identical columns (data fields) for every row (records). Much of the software produced at this time for use with factual data is now thought of as inadequate because of its inability to handle the structural complexity of the data (Allkin, 1984) and has been overtaken by enormous developments in database software. What has survived are the cases where a single large file was all that was needed. The most notable examples are the large bibliographic files. Many of these hold enormous quantities of legume citations and are in regular use today as bibliographic sources: BIOSIS PREVIEWS, CAB, AGRICOLA, AGRIS and others (Lockerman et al., 1987; Bisby, 1985).

Specimen-based systems

Legumes comprise a major component in a number of general botanical specimen-based systems, such as the curatorial system PRECIS, which records all of the specimen label data from the Pretoria herbarium, or the RBG Kew's Botanical Garden system with accession data on plants cultivated at Kew. More particular specialization in legumes is found in many of the germplasm databases: legumes tend to be a major component of general germplasm systems, such as the GRIN (Genetic Resources Information Network) system run by the U.S.D.A. (U. S. Department of Agriculture), and the Commonwealth Forestry System run by the Commonwealth Forestry Institute in Oxford. Additional institutions specialize in legume germplasm or legume cultivars and consequently have systems largely devoted to them—the international centers with particular responsibilities for legume crops, such as CIAT *(Phaseolus),* ICARDA *(Vicia faba* and *Lens),* ICRISAT *(Cajanus*

cajan), and national institutions with crop responsibilities (e.g., the U.K. *Pisum* database at DAFS, Edinburgh, U.K.).

Perhaps the most notable germplasm systems for legume workers are the databases established by some crop breeding research groups to incorporate copies of all of the known germplasm databases on a particular crop side by side in one multi-file database for comparison purposes. The examples I know of are: the World Pea Germplasm Database—John Innes Research Institute, Norwich, organized by Dr. P. Matthews, and the World *Phaseolus* Germplasm Database at the Faculté des Sciences Agronomiques de L'Etat, Gembloux, organized by Prof. Marechal. Both allow sophisticated searching and comparison of records. One can locate duplicate accessions in different genebanks; examine the ranges of variation known in the different collections; and search for particular gene combinations, either for breeding purposes or in pursuit of gene-mapping studies. Where evaluation data is present for duplicated accessions, one can even compare the different evaluations of the same accession under different conditions in different continents (Lockerman et al., 1988).

It is important to recognize the distinction between the data held in a germplasm system and that held in a taxon-based system. The germplasm system will typically record thousands of germplasm accessions in one crop species. The data held will include "passport data," showing its identification and origin, and "evaluation data," showing differences among the accessions in features thought valuable in the plant breeding community. But more general elements of botanical information are missing. One would not normally be able to glean any nomenclatural or taxonomic information. Interspecies variations, such as characters used for identification, would be absent, and a summary of the range of variation recorded, while valuable to a breeder, would not give what other botanists would consider a useful description of the species and its variation.

NAME-ORIENTED SYSTEMS

A technical task of great importance to taxonomy is listing and indexing names, as has been carried out in the past by the compilers of *Index Kewensis* and the Gray Card Index. Because such indexes are used by taxonomists on whose work other scientists depend, much attention is paid to the accuracy of details, such as the validity, publication place and date, authority, status, orthography, and typification of a name. It is important that taxonomists should get the details right, but these details are not of direct usage by other legume researchers. Legumes are again a major component of some general systems and the focus of other specialized systems. The most notable general system is the Missouri TROPICOS system, which presently lists entries and nomenclatural details of 65,000 legume names and gives on-line access to taxonomists working at the Missouri Botanical Garden.

Legume biologists should not confuse name-oriented systems, such as TROPICOS or *Index Kewensis,* with taxon-oriented systems. In their raw state the name-oriented systems consist of catalogs of names, most of which are synonyms and not the accepted names of taxa. As such they provide primarily an internal service within the profession for taxonomists. But the intention with TROPICOS is subsequently to use it as the core of a taxon-oriented system containing other botanical data. To do this one has to identify which of the taxonomic names are actually the accepted names of taxa. An example is the nomenclator of legume generic names (Gunn, 1983). Gunn has marked against each name its status according to the Polhill & Raven systematic survey and so is able to sort the names and produce a taxon-based checklist of accepted genera.

TAXON-ORIENTED SYSTEMS

The taxon-oriented systems are expected to provide the factual information systems suitable for use throughout botany in the future. They contain information about taxa, usually species or

cultivars, which are the units about which the majority of the botanical information of interest to biologists is recorded.

Taxon-oriented systems are also the place where the taxonomy/botany interface occurs. Taxonomists decide and publicize which species exist: other botanists study these species and accumulate scientific knowledge about them. Hence the core of a taxon-oriented system, the species (or cultivar) checklist, tends to be the end-point of a taxonomic project and the starting point for other botanical activities.

Given the difficulty of covering all Angiosperms, taxon-oriented systems, like traditional botanical books, tend to be either *floristic*, covering all plants of a certain area or domain, or *monographic*, covering plants of a particular taxon on a worldwide basis. Given their importance, I shall examine a few examples in further detail.

MONOGRAPHIC SYSTEMS

The first functioning taxon-based plant databases were monographic systems established by Watson for genera of grasses (Watson & Dallwitz, 1980) and the genera of Caesalpinioideae (Watson, 1981). Both of these consisted of descriptive data, primarily morphology and anatomy, entered for each of the large sets of genera. They use the CONFOR program of Dallwitz (1980) in which descriptive data is held in DELTA format and then communicated to a set of programs that can print descriptions and keys. Pankhurst has also adopted the DELTA format for his suite of identification programs so that these can be used alongside those of Dallwitz, for instance, to provide on-line identification facilities. The CONFOR program does not, however, provide normal retrieval facilities, the sort of on-line searching one associates with database usage, and it lacks a taxonomic checklist, hierarchical structure, and cross-indexing to synonyms. Dallwitz and Pankhurst continue to develop programs that use the DELTA format—there is now a PC version for MS-DOS microcomputers and plans for a retrieval facility (Pankhurst, pers. comm.).

The Caesalpinioideae generic database contained 71 characters for general morphology and 45 characters of anatomy, chemistry, and pollen ultrastructure in 1981 when the full set of descriptions was printed out in a microfiche published with Watson's 1981 paper. The publication of the complete database printed out on microfiche seemed an excellent way of distributing the information economically. On the microfiche one can read the comparative data for each genus.

A further development of the monographic taxon-based systems was the Vicieae Database (Adey et al., 1984; Bisby, 1984c; Babac & Bisby, 1984) providing descriptive geographical and chemical data for the 320 species of the tribe Vicieae. This project deliberately experimented with an information service that might serve the general scientific community; the compilers used species as the unit taxon and incorporated data on geographical distribution in addition to morphology and phytochemistry. The Vicieae Database was one of the first botanical systems to incorporate data retrieval capabilities so that, for instance, the operator could extract the species with the pigment Malvidin present in the petals and for these species print the full scientific name and the presence or absence of other pigments. It also contained a nomenclatural system that was used to produce checklists with optional synonymy (Allkin et al., 1986). By linking the database with a germplasm collection it was often possible to follow up factual inquiries with supplies of the plants that users wished to investigate. The extra database facilities were, however, achieved by linking together a range of programs: SYNONYMS (by R.J. White), EXIR (by R. Brill & G.F. Estabrook), CONFOR (by Dallwitz), ON-LINE (by Pankhurst), and consequently the "database" was an awkward affair, requiring skilled operation and precluding on-line usage by the public. It attracted considerable enthusiasm from a trial users panel (the Vicieae Database Study Group) and showed that a monographic database is feasible and that a broader information service to the scientific community would be welcomed.

The Vicieae Database (Bisby, 1986) continues to be developed and is now being used as a pilot

scheme for the larger ILDIS (International Legume Database & Information Service) and in a collaborative project with BIOSIS to set up an experimental on-line information service—the Vicieae Database Online at BIOSIS—which went on trial in August 1987. The Southampton Laboratory is also developing improved software: Allkin and Bisby are working with Winfield in Edinburgh on the ALICE Biological Diversity Database System for MS-DOS microcomputers; and Allkin, White, and Bisby are working on the BAOBAB taxonomists' data handling system.

FLORISTIC SYSTEMS

Despite two of the earliest taxonomic database projects being floristic ones (*Flora de Veracruz* and *Flora of North America*), little progress was made with floristic databases until the 1980s. This is probably because the task is more difficult in several ways than for a monographic database. First, the needs for a checklist, for synonymic cross-indexing, and for knowledge of the taxonomic hierarchy are immediate: the early monographic systems were one-level systems for genera, species, or cultivars and were able to operate without these. Second, establishing simple comparative or homologous descriptive characters is more demanding for the plants of a whole flora than for species or genera of the same higher taxon (Pankhurst, 1983).

So the arrival of taxon-based floristic databases has coincided with the arrival of the more complex multi-file relational database management programs. At the "taxonomic" end of the spectrum we have the ESFEDS database covering the checklist, synonymy, hierarchy, and additional fields for taxa in *Flora Europaea* (Derrick et al., 1986). At the more applied end, there are the IUCN databases covering aspects of all reported rare and threatened plants (Mackinder, 1984), and the SEPASAL database at Kew, covering plant uses for arid lands. Both contain a checklist and synonymy including many legumes: neither contains descriptions.

GLOBAL SYSTEMS

Progress in database organization and communications makes it now technically feasible to build a database to cover all of the world's plants. I know of no solid progress with a global plant system, but we should note that many of the organizers of large databases of international significance are taking part in the annual meetings of the international *Taxonomic Databases Working Group for Plant Sciences*. This group has as its aims to investigate the possibilities for standardization and cooperation (Bisby, 1986, 1987).

III. ILDIS (International Legume Database & Information Service)

The ILDIS database is an example of a monographic taxon-oriented system. As it is wholly devoted to legumes, I shall describe it in some detail.

ILDIS is a cooperative project to establish an International Legume Database & Information Service. It has its roots in three earlier activities. The proposal for such a project arose at the evaluation seminar for the Vicieae Database Project at Southampton University: trial users and advisers asked if the concept of the Vicieae Database could not be developed to make a more significant impact on botanical information provision. Second, a human network of communications and cooperation among legume biologists already existed, the network set up by Polhill at Kew to provide a systematic and economic review of all legumes for the 1978 International Legume Conference. Third, it was discovered in 1984 that numerous research groups had plans for developing databases on particular groups of legumes, of which the largest was the New World Legumes

Database project at the Missouri Botanical Garden: a move toward collaboration seemed advantageous to all.

The prime purpose of ILDIS is to provide an international information service for the world's legumes. It should serve the full range of scientists, professionals, and international agencies that work with legumes. The main elements will be a database, an information service, and an electronic communications network.

The database is taxon oriented and will comprise a checklist of all known legume species and will hold different types or depths of information for each species. All species will be recorded for ILDIS Type 1 data—geographical distribution and general knowledge data. About one-third of all species, those in tribes selected for economic or academic priority, will, in addition, have more detailed ILDIS Type 2 data on economic importance, husbandry, botanical features, phytochemistry, breeding, and germplasm sources. The database will be made available internationally by distribution (with appropriate software) on disc, by an on-line service, and by conventional communications from an inquiry center.

The communications network presently links just the research groups creating the database. It will shortly be extended to link with legume breeding, biotechnology, and product development laboratories and with germplasm and plant exploration projects. Subsequently the network will be integrated with the on-line database service so that users can query the database and use the network to contact listed experts, projects, germplasm sources, and other databases.

The ILDIS Project has been established with some care as an international cooperative program open to all. Participating research groups elect both an international board responsible for decisions, and two specialist groups. Of these, the Data Content Group is responsible for the botanical content of the database and the Software & Computing Group sees to the computing policy. As ILDIS Project Coordinator, I am responsible to the Board for the day-to-day management of the project, assisted by two other members of the ILDIS Executive—Dr. R.M. Polhill at Kew and Dr. J.L. Zarucchi at Missouri.

ILDIS DATABASE—PHASE I

The first phase of establishing the database is to create the checklist and Type 1 data for all known legume species, about 17,000 species in all. This is now in hand as a two-stage process: first, creation of regional checklists by continents or large regions and, second, the taxonomic editing and amplification of general knowledge data by specialist laboratories.

For each continent or large region a "checklist compiler" at an institution specializing in the flora of the region is compiling the species checklist using the existing literature (floras, checklists, and monographs) to obtain accepted species names and geographical distributions. Where the data are available to the compiler, further Type 1 data fields are filled as well. The result will be a series of checklists covering all of the world's legumes but suffering in places from inconsistent taxonomic treatment and in the duplicate entry of species spanning several continents. The lists will then be merged and passed to monographic experts for editing and amplification so that, as far as is possible, the finished ILDIS Type 1 Database will be taxonomically consistent, of high quality, and free of duplications.

For each tribe (or in some cases part-tribe or large genus), ILDIS has contacted experts to help edit the Type 1 data. For the selected tribes of economic or academic importance, ILDIS has enlisted an expert to act as taxonomic coordinator for the tribe. The taxonomic coordinator takes responsibility for drawing in other expert opinion, not only for editing the checklist for taxonomic consistency in the tribe and amplifying the Type 1 data, but also for a continuing project to update the data and taxonomy of the tribe and subsequently to assist in collating Type 2 data.

The precise definition of Type 1 data fields has been undertaken at the ILDIS Coordinating Centre at Southampton in consultation with the Data Content Group. The thirteen fields (see Table

TABLE 1. ILDIS Type 1 data fields for Checklist, Geography, and Common Knowledge data for all legume species. The references are selected where possible from widely available publications and are held in a single bibliography.

Data area	Field numbers	Fields	Numbers of entries per taxon
Checklist	1	Accepted name, authority, and reference (or provisionally accepted name)	1
	2	Synonym, authority, and reference	0–many
	3	Tribe and code (or subtribe and code)	1
Geography	4	Geography with references (status in each country)	1–many
Common knowledge data	5	Vernacular name and reference	0–many
	6	Vegetation type per continent, with reference	0–many
	7	Life form (whether perennial, whether herb/shrub/tree, whether climbing)	1
	8	Conservation status (global) and references	1
	9	Importance to man and references	0–many
	10	Notes about the taxon	0–many
	11	Reference to a published description	0–few
	12	Reference to a published illustration	0–few
	13	Reference to a published map	0–few

1) are summarized in one document (Hollis, 1986) and defined in detail in another (Hollis, 1987). The only area of Type 1 data still open to debate is the exact treatment of national territories and the status of a species within these: this is the subject of continuing discussion by the Data Content Group and of efforts to agree on a system to give compatibility with other international projects taking part in the international Taxonomic Databases Working Group. When finalized, a third document will detail the system of continents, countries, and areas being used by ILDIS, and the internationally agreed PECS (Plant Existence Categorization Scheme) system for recording a plant's status in these areas.

A software system called ALICE has been developed by Winfield, Allkin, & Bisby for entering, checking, editing, and viewing Type 1 data. The design and interface is based on a relational file structure that reflects advice given by the ILDIS Software & Computing Group, Allkin and White's work on the BAOBAB software design, discussion with Polhill and Hollis on the definition of Type 1 data, and feedback from beta-testing by Lock. The file structure is not tied to any one software implementation. The implementation carried out by Winfield meets the requirement by the Software & Computing Group that it must be usable on microcomputers of the type widely available around the world—those PCs/XTs/ATs and clones with PC-DOS or MS-DOS as operating systems—so that a variety of checklist compilers and taxonomic coordinators can use the same software. ALICE is also more general than was needed by ILDIS so that it can be used for many other projects. The result is a satisfactory database management system that is menu-driven, easy to use, and reasonably foolproof because of many data consistency checks. It is being installed in laboratories as widely dispersed as Huhhote City in Inner Mongolia and Villavicencio in Colombia.

The African, European, and American regional datasets were completed in 1988, and the present plan is to release the combined Type 1 Database for public use by 1991. Three different types of service are planned. One is to distribute the database with software, possibly on a CD-ROM disc. Another is an experimental on-line service being sponsored by BIOSIS. And the third is to operate a conventional inquiry center probably in a tropical or developing country for the many legume workers who do not have microcomputer or communications facilities.

ILDIS COMMUNICATIONS NETWORK

ILDIS plans to establish an international electronic communications network that will allow individual legume workers to contact experts; to contact legume projects in germplasm storage, plant exploration, biotechnology, and plant breeding; and to gain access to the on-line ILDIS and possibly other databases. Like the on-line database, this communications network is sponsored by BIOSIS. A preliminary experimental system linking the ILDIS participating laboratories and members of the Vicieae Database Study Group was launched in March 1987 as a closed user group using the BTGold DIALCOM services. Further development will involve opening this network to users so that they can contact the project's growing list of experts by electronic mail.

ILDIS DATABASE PHASE II

Planning is now proceeding for the second phase of ILDIS, extension of the database to cover more detailed information on the economic utilization, botany, and chemistry of selected tribes. Work by the ILDIS Data Content Group is going into defining the Type 2 data in such a way that the data can initially be collected for just the tribes selected for their economic or other interest, but subsequently might gradually be extended to all legume tribes. Preliminary versions have the following principal headings:

1. Husbandry & Products (for food, forage, fuel, forestry, etc.)
2. Germplasm Sources
3. Plant Improvement & Biotechnology
4. Breeding Data
5. Nodulation & Nitrogen Fixation
6. Phytochemistry
7. Ecology
8. Brief Morphological Description

There remains much to be done in planning the assembly of this information and in designing this part of the database. Several principles seem clear—ILDIS will continue soliciting participation on a cooperative basis, the center of gravity of ILDIS will gradually move from taxonomic botany into applied botany, and many of the laboratories collating Type 2 data will use the first phase ILDIS Database as the backbone to which data will be added. It is intended that the tribe coordinators will organize much of the purely botanical data, and other specialist laboratories will collate, for example, classes of phytochemical or biotechnology data.

The preliminary list of 18 tribes/subtribes selected for detailed coverage with Type 2 data (Table 2) shows the nine groups selected for their economic importance, and the other nine groups selected for the interest in them for minor crops, ornamentals, importance in the evolution of legumes, or ecological significance. The enormous and important tribe Phaseoleae is being treated as several subtribes taken separately.

LEGUME RESEARCH AND COLLABORATION

While ILDIS is primarily an information and communications project, it should be stressed that it is intended to stimulate the study and the utilization of legumes in a very wide range of disciplines. Much of this botanical study and plant development research will be carried out by laboratories participating in ILDIS. One example is the compilation of the taxonomic checklist: Polhill & Raven (1981) contains a comprehensive survey of legume genera, but the ILDIS checklist will be the first attempt by taxonomists at a consistent enumeration and study of all known species. Similarly, many

TABLE 2. Preliminary list of groups of legume species selected by ILDIS for detailed coverage in Type 2 data.

LEGUME GROUPS SELECTED FOR ECONOMIC IMPORTANCE		
	MIMOSOIDEAE	
Acacieae	Gum arabic and other gum-producing acacias	*(Acacia)*
Leucaena (Mimoseae)	Multipurpose trees in *Leucaena*	*(Leucaena)*
	PAPILIONOIDEAE	
Phaseolinae (Phaseoleae)	Common beans and relatives	*(Phaseolus, Vigna, Macroptilium, Lablab, Psophocarpus)*
Cajaninae (Phaseoleae)	Pigeon pea and relatives	*(Cajanus)*
Glycininae (Phaseoleae)	Soybeans and relatives	*(Glycine)*
Trifolieae	Clovers and medics	*(Trifolium, Medicago, Trigonella)*
Vicieae	Vetches and peas	*(Vicia, Lathyrus, Lens, Pisum)*
Cicereae	Chickpeas	*(Cicer)*
Genisteae	Lupines, gorses, and brooms	*(Lupinus, Ulex, Cytisus)*
OTHER SELECTED LEGUME GROUPS		
	CAESALPINIOIDEAE	
Caesalpinieae, Amherstieae, Detarieae		
	MIMOSOIDEAE	
Calliandra (Ingeae)		
	PAPILIONOIDEAE	
Sophoreae, Dipterygeae, *Astragalus* (Galegeae), Desmodieae, Indigofereae		

organizations specialize in legume germplasm: but when the germplasm entries are available in ILDIS Type 2 data, not only will it be possible to locate specialist germplasm collections for most genera, but we will be able to know precisely which groups are poorly covered. It may then be possible to stimulate either the legume staff at the Kew Genebank, IBPGR, or other specialists to fill the gap, and this in turn may lead to more extensive and more accurate seed provision for wide breeding programs and for phytochemical analysis.

IV. WHAT SHOULD WE EXPECT IN THE FUTURE?

The organizers of this conference particularly asked me to speculate on future developments in information systems in relation to legume research. What will things be like in five or ten years? Before we allow our imaginations to run off with rosy pictures of high-tech facilities available to every botanist at the flutter of the finger-tips, let us be realistic about what is happening now. As a generality, we botanists are presently five to ten years behind the leading edge of information technology, and if anything, the gap is widening. Botanical information systems are poorly funded—microcomputers are only now (1989) reaching every laboratory, and relational databases have yet to arrive. But more importantly, we seem not yet to have learned the lessons learned by bankers, finance, and business: it is software that we need. Designing databases, tutors, models, expert systems; implementing and testing them; providing user-friendly interfaces; maintaining,

distributing, and documenting them—all require work to be measured in man-years with costs far in excess of the hardware costs. This work can be minimized if we focus professional-quality work on preparing a few high-quality software packages of general application that can be used at many sites. I would cite Pankhurst's on-line identification programs (Pankhurst, 1978), and the ALICE program as examples. It is salutory to say that I know of only one "botanical" organization that provides adequately for its own needs, and even that organization has developed specialist software for itself rather than generalized software for distribution.

ARTIFICIAL INTELLIGENCE

Despite the fuss about expert systems and the often-quoted examples of their potential in plant identification, quite good computerized identification systems have been available since the 1970s (see Pankhurst, 1976, 1978). Although the name wasn't used then, these are special-case expert systems in that they are rule-based and can explain their conclusions in terms of the rules (a rare case of us being "ahead" !). But modern expert systems undoubtedly will provide improvements in ease of adjustment of the data; in ease of use; and in systems that "learn." These learning systems will monitor their own success rate and decide which identification routes through a polyclave give the best results. So they will adjust for the difficulty of correct observation and the difficulty of interpretation associated with each characteristic.

Few identification programs presently depend on probabilistic calculations—that it is most likely to be species X and not Y because, among the species that can have this character combination, X has it far more frequently or is more frequently encountered than Y. Such additions may improve identification uses of expert systems and teaching uses where one will use an expert system to teach "lessons" about the recognition of plants. An example of this occurs in my own teaching to undergraduates on a field course. The object is to teach students to recognize the major families of Angiosperms. In the first few days we concentrate on large numbers of species from just a few families, familiarizing the students with explicit recognition features and the subconscious "recognition" of unstated features. But the explicit recognition features given at first (e.g., "fruit a dehiscent pod," "5 petals—1 standard, 2 wings, 2 keel petals") are in fact only good enough to identify some but not all members of a family and only distinguish a small subset of the world's families. But it works, and students get a grasp of just this small, common subset before extending their range of families and realizing the frequency of exceptions. Similar strategies can be used in expert systems—how much better to start people with this than the keys to all European plants!

A formidable but fascinating idea is to use rule-based expert systems to study, test, or improve the international codes of nomenclature. As everyone knows, it is surprising, considering the length and detail of the codes, that cases still occur where either the codes make no adequate ruling or where two rules come into conflict. Workers at the BM(NH) (R. Eastward, pers. comm.) and at RBG Kew (C.H. Stirton, pers. comm.) have plans to build such systems. Similarly, designers of "taxonomically intelligent" databases (R. Allkin & R.J. White, pers. comm.) can describe nomenclatural and associated taxonomic linkages that seem impossible to program adequately because of logical loopholes. The vision here is of an ICBN expert system applied to a database of the complete *Index Kewensis*. The validity and legitimacy of any name could be tested, new rules checked for consistency with the main code, and the nomenclatural consequences of a rule change predicted by determining how many names would be affected. But, alas, despite its increasing importance, systematic botany is poorly funded and I am not optimistic that this formidably difficult project will be realized.

The most striking advances from artificial intelligence will be seen by legume workers in the user-friendliness and apparent intelligence (one should say reduced stupidity!) of two classes of software—databases and expert system/modelling programs. Much progress has already been made with the use of tailored menus. The application of *intelligent front end* and *natural language and*

specially tailored interfaces will mean that conversations with these programs, giving of instructions, checking for detail, and production of results will all be that much easier for inexperienced humans. "Tell me everything about vetches," or "When should I spray my soybeans?" will become acceptable commands with responses such as "Names and distribution first, then morphology if you wish—O.K.?" and "Are we talking about insecticides, fungicides, or herbicides?"

THINK BIG—THINK SMALL

Arguments are put forward increasingly frequently that we should "think big" in terms of international botanical organization, communications, project organization, software usage, and software, hardware, and data standards. But at the same time we should "think small" in terms of hardware and software, delivery of services, use of facilities, data-entry, and other benefits that we want to come to every individual laboratory, onto every botanist's desk on cheap standard equipment wherever possible.

Let us apply the philosophy at the hardware level. If we amass databases, expert systems, or modelling systems in large, often institutional mainframe computers, then users outside the immediate computing center must gain access by telecommunications links. The equipment needed (terminal, modem, wiring, telephone-line installation) is typically similar to or slightly less than the cost of personal microcomputers. But the telecommunications are technically troublesome, and the running costs are large (although falling, possibly rapidly with the advent of fiber optics). An alternative is possible and advantageous in some contexts—for users to obtain microcomputers and to transfer the data and software that they need most to their own machine. The same or only slightly greater capital expenditure can be rewarded by fewer technical troubles and lower running costs. However, the smooth running of the machine depends on a higher level of organization. It is terribly important to buy a standardized microcomputer, standardized software, a standardized printer, discs, and so forth.

How does it work at the software level? The difficulty here is that software preparation is time-consuming and expensive, and software is usually specific to a particular language or computer operating system, sometimes to one particular machine configuration. So if we were choosing between preparing software for a few mainframes or thousands of *different* microcomputers we would choose the former. But, by some quirk of dynamics in mass marketing, the operating systems and languages on mainframes are not standardized (mostly each company has its own combination), whereas on some microcomputers they are. Therefore, in some contexts it is easier to provide botanists in many countries with access to the same software by distributing standard software for a standard microcomputer than it is to set the same software up on fewer mainframes but of different types.

IMAGES—COMPUTER GRAPHICS AND VIDEO

A third major development in botanical information systems will undoubtedly arise through the widespread use of image technology. It is already commonplace for medium- or high-resolution graphics facilities to be provided with the mass-produced microcomputers (Figs. 1, 2). I know of a number of projects investigating the use of either vector-based graphics or pixel-based graphics to hold images of plants for use in taxonomic databases or in identification systems: we are just starting one of these as an addition to our Vicieae Database. What an improvement we should see in computer-assisted identification when bold line drawings, not words, appear in the windows of our screens as in the windows shown in Figure 1 (Hawthorne, 1985). Others are using these images for analytical techniques.

A second group of image projects are using computer-driven videodisc apparatus. Again, there

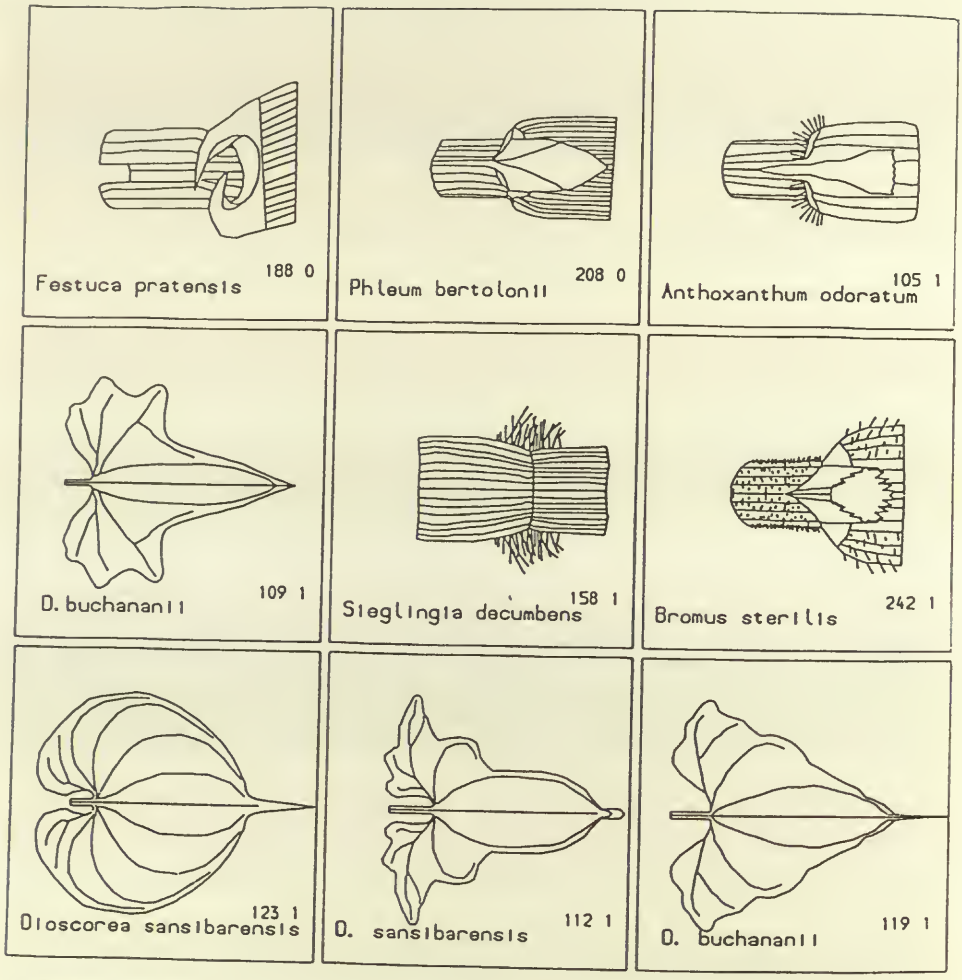

FIGURE 1. Sample of vector images, reproduced with permission from Hawthorne (1985). Numbers refer to number of vectors (followed by '0') or half the number of vectors (followed by '1') for symmetrical shapes.

are many projects—the one I know of, the Nottingham University IBIS (Interactive Biological Information System), involves the use of interpretive software, for education, taxonomic study, identification, and other functions. This software controls access to numerous video images of plants and animals held on a generic video disc.

CONCLUSIONS

Databases and electronic information systems are now beginning to make a significant contribution to the information available to legume researchers.

The ILDIS (International Legume Database & Information Service) project plans to provide a database and communications network expressly directed at the legume research community. The project seeks the cooperative participation of specialist research groups throughout this community.

FIGURE 2. High-resolution (c. 1000 × 800) monochrome image of a complex line drawing *(Craibia brevicaudata)*.

There is much more as yet to come from the information technology revolution—artificial intelligence, electronic communications, international networks, image systems, and above all, modern software, to facilitate improved information provision, communication, and analysis. And we botanists have to go a lot further, particularly in investing in system design and in software, before dreams of a high-technology information-rich research community are realized.

ADDENDUM. This paper was prepared in 1986. Much has happened since then, in particular: (i) There has been much development of TROPICOS, with its recent association with the community involved in the Flora

of North America project. Presumably additions will be made to the design so that a taxon-oriented view will be made available to users.

ii) There has been steady progress with the ILDIS system, which in September 1989 contained Type 1 data for some 13,500 legume taxa.

iii) The MS-DOS-based ALICE software used by the ILDIS project has taken on a life of its own. The software is now available for general use in species diversity databases and checklists for plants and animals, with many of the data fields being named and defined by the user.

iv) The Taxonomic Databases Working Group has agreed on standards for names in taxonomic databases, for world geography recording units, for DELTA descriptions and for plant status records. XDF, the exchange data format, has now been used for data exchange between several different systems.

v) There is now active consideration for one or more global systems, and an international symposium is being held in autumn 1990 entitled "Designs for a Global Plant Species Information System."

vi) A number of botanical computer systems now in place do incorporate graphics representations, and others are now available as videodiscs. Very shortly these will include systems of relevance to legume research—image and data sources plus aids to identification.

References

Abbott, L. A., F. A. Bisby & D. J. Rogers. 1985. Taxonomic Analysis in Biology: Computers, Models and Databases. Columbia Univ. Press, New York.

Adey, M. E., R. Allkin, F. A. Bisby & R. J. White. 1984. The Vicieae Database: an experimental taxonomic monograph. Pp. 175–188 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

Allkin, R. 1984. Handling taxonomic descriptions by computer. Pp. 263–278 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

———, D. J. Goyder, F. A. Bisby & R. J. White. 1986. Names and synonyms of species and subspecies in the Vicieae: issue 3. Vicieae Database Project Publication No. 7.

Babac, M. T. & F. A. Bisby. 1984. A chemotaxonomic database. Pp. 209–218 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

Bisby, F. A. 1984a. Information services in taxonomy. Pp. 17–33 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

———. 1984b. Automated taxonomic information systems. Pp. 301–322 *in* V. H. Heywood & D. M. Moore (editors), Current Concepts in Plant Taxonomy. Academic Press, London & Orlando.

———. 1984c. The Vicieae Database: products and services. Webbia 38: 639–644.

———. 1985. Plant information services for economic plants of arid lands. Pp. 413–425 *in* G. E. Wickens, J. R. Goodin & D. V. Field (editors), Plants for Arid Lands. George Allen & Unwin, London.

———. 1986. Plans for an International Legume Database (ILD). Pp. 22–24 *in* A. K. Kaul & D. Combes (editors), *Lathyrus* and Lathyrism. Third World Medical Research Foundation, New York.

———. 1987. Report of the second meeting of the Taxonomic Databases Working Group for Plant Sciences. [Unpublished report available from F. A. Bisby, Southampton University, or from R. W. Kiger, Hunt Institute, Pittsburgh.]

Brill, R. C. & G. F. Estabrook. 1984. Management of almost flat files in systematic biology using TAXIR. Pp. 53–67 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

Dallwitz, M. J. 1980. A general system for coding taxonomic descriptions. Taxon 29: 41–46.

Derrick, L. N., V. H. Heywood & J. Van Scheepen. 1986. Databases and infraspecific taxa—the role of the European Taxonomic Floristic and Biosystematic Documentation System. Pp. 77–86 *in* B. T. Styles (editor), Infraspecific Classification of Wild and Cultivated Plants. Oxford Univ. Press, Oxford.

Gunn, C. R. 1983. A nomenclator of legume (Fabaceae) genera. Tech. Bull. U.S.D.A. 1680: 1–224.

Hawthorne, W. D. 1985. Images in Botanical Databases. Report available from Biology Dept., University of Southampton, U.K.

Hollis, S. 1986. ILDIS Type One data: details of information in the Type One data fields. ILDIS Publications, Southampton.

———. 1987. ILDIS Type One Data: summary. ILDIS Publications, Southampton.

Lockerman, R. H., F. A. Bisby & L. J. G. van der Maesen. 1988. Workshop: Integration of information on

plant diversity. Pp. 129–133 *in* R. J. Summerfield (editor), World Crops: Cool Season Food Legumes. Kluwer, Dordrecht, Boston & London.

MACKINDER, D. C. 1984. The database of the IUCN Conservation Monitoring Centre. Pp. 91–102 *in* R. Allkin & F. A. Bisby (editors), Databases in Systematics. Academic Press, London & Orlando.

PANKHURST R. J. 1976. On-line identification program. Internal document from the author. British Museum (Nat. Hist.), London.

_____. 1978. Biological Identification. Arnold, London.

_____. 1983. The construction of a floristic database. Taxon 32: 193–202.

POLHILL, R. M. & P. H. RAVEN (editors). 1981. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

WATSON, L. 1981. An automated system of generic descriptions for Caesalpinioideae, and its application to classification and key-making. *In:* R. M. Polhill & P. H. Raven (editors), Advances in Legume Systematics. [Pp. 65–80 in Part 1 plus microfiche in back cover of Part 2.]

_____& M. J. DALLWITZ. 1980. Australian Grass Genera—Anatomy, Morphology and Keys. The Australian National University Research School of Biological Sciences, Canberra.

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The following entries are provided for subjects, legume genera and species, and associated organisms as treated in the 35 papers contained herein. In cases where a genus/species or subject is mentioned numerous times in a particular chapter, only its first occurrence in that chapter is noted. Subject entries are mostly under rather broad headings leading the reader to one or more of the chapters where that topic is considered (e.g., many subtopics under pollination are not indexed in the Schrire paper). Tables and figures in this volume are not indexed.

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